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Effects of pH, Temperature and Salinity on Age 0+ Atlantic Whitefish (*Coregonus huntsmani*) with Implications for Recovery Potential

Effets du pH, de la température et de la salinité de l'eau sur le corégone de l'Atlantique (*Coregonus huntsmani*) d'âge 0+ et incidences sur le potentiel de rétablissement

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ABSTRACT

Endemic to Canada, the anadromous Atlantic whitefish only occurs in Nova Scotia, and spawning runs have only been documented in two watersheds. Since 1982, the global distribution of Atlantic whitefish has been limited to the Petite Rivière watershed, where a land-locked population persists within three small semi-natural lakes that cannot be accessed from the sea. Atlantic whitefish were designated as endangered by Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1984 and 2000; in 2006, a Recovery Strategy (DFO 2006) was developed which has the goal:

> *"to achieve stability in the current population of Atlantic whitefish in Nova Scotia, re-establishment of the anadromous form, and expansion beyond its current range."*

In order to acquire information to help guide recovery activities, a series of lab-based experiments were initiated in 2004 to assess the response of early life stages of Atlantic whitefish to water pH, temperature and salinity. This document describes the experiments and reports the outcomes. The results are then applied to available water chemistry data for rivers in Nova Scotia's Southern Upland (SU) region lying within the historical range of the species to assess recovery feasibility under a series of plausible life history scenarios.

The results show that Atlantic whitefish are tolerant to full seawater at early stages of development (larvae-juvenile) and exhibit increasing tolerance to pH through ontogeny such that:

Eggs<hatch<larvae=early juveniles<juveniles

Model simulations show freshwater resident populations can potentially survive in all watersheds in the SU region of Nova Scotia, as river specific median survival probabilities ranged from 0.20 to 0.96, with reduced survival occurring in the most acidified rivers. The inclusion of anadromous migrations into the simulations resulted in 30% increases in survival probability for Atlantic whitefish in the most acidified rivers, irrespective of the life stage in which the migration occurs.

RÉSUMÉ

Espèce endémique du Canada, le corégone de l'Atlantique anadrome se retrouve seulement en Nouvelle-Écosse, et des montaisons n'ont été documentées que dans deux bassins hydrologiques. Depuis 1982, la répartition mondiale des populations de corégones de l'Atlantique a été limitée au bassin de Petite Rivière, dans lequel une population confinée aux eaux intérieures demeure dans trois petits lacs semi-naturels qui ne sont pas accessibles par la mer. Le corégone de l'Atlantique a été désigné « en voie de disparition » par le Comité sur la situation des espèces en péril au Canada (COSEPAC) en 1984 et en 2000; en 2006, une stratégie de rétablissement (MPO 2006) a été élaborée. Son but est le suivant :

> *« atteindre une stabilité de la population existante de corégone de l'Atlantique en Nouvelle-Écosse, le rétablissement de la forme anadrome et l'expansion hors de l'aire de répartition actuelle. »*

Afin d'obtenir des renseignements pour orienter les activités de rétablissement, une série d'expériences en laboratoire a été entreprise en 2004 pour évaluer la réaction des stades précoces de l'existence du corégone de l'Atlantique au pH, à la température et à la salinité de l'eau. Ce document décrit les expériences et présente les résultats. Ces derniers sont ensuite appliqués aux données disponibles sur les propriétés chimiques de l'eau des rivières de la région des hautes-terres du sud de la Nouvelle-Écosse située dans les aires de répartition historiques de l'espèce afin d'évaluer la possibilité de rétablissement en fonction d'une série de scénarios de cycle biologique plausibles.

Les résultats indiquent que le corégone de l'Atlantique tolère l'eau de mer aux stades précoces du développement (stade larvaire, juvénile) et démontrent une tolérance croissante au pH dans l'ontogénie :

Oeufs<éclosion<larves=stade précoce juvénile <juvéniles

Les simulations de modèle démontrent que les populations résidant en eau douce peuvent possiblement survivre dans tous les bassins hydrologiques de la région des hautes-terres du sud de la Nouvelle-Écosse, étant donné que les probabilités moyennes de survie dans les rivières sont de 0,20 à 0,96, avec une survie réduite dans les rivières les plus acidifiées. L'inclusion des migrations anadromes dans les simulations a eu pour résultat une augmentation de 30 % de la probabilité de survie du corégone de l'Atlantique dans les rivières les plus acidifiées, peu importe l'étape du cycle de vie lors de la migration.

INTRODUCTION

Atlantic whitefish, *Coregonus huntsmani*, belong to the family Salmonidae, and are part of the subfamily Coregoninae, which has a global distribution across the northern temperate and sub arctic zones (Lindsey and Woods 1969). Atlantic whitefish are both phylogenetically (Bradford et al. 2010) and phenotypically (Hasselman et al. 2009) distinct from all other coregonid species. They represent a unique lineage within their genus (Bradford et al. 2010).

Endemic to Canada, Atlantic whitefish occur only in Nova Scotia where they are thought to have been wide-spread prior to European colonization (Bradford et al. 2004a). By the mid-twentieth century, reported occurrences were limited to the Tusket-Annis Rivers and Petite Rivière watersheds (Figure 1), as well as rare incidences in coastal waters adjacent to these two river systems. Reported occurrences since 1982 have been limited to the Petite Rivière watershed where a land-locked population persists within three small semi-natural lakes that cannot be accessed from the sea (Bradford et al. 2004a). Atlantic whitefish have been designated since 1984 as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). They are considered to be at risk of extinction because of habitat loss and degradation caused by several factors including acidification (acid rain), water level fluctuation, ineffective fish passage around dams and introductions of exotic species. Atlantic whitefish have been protected under the federal *Species at Risk Act* (*SARA*) since 2004 (DFO 2006). In compliance with Section 37 of *SARA*, Fisheries and Ocean Canada (DFO) has developed a Recovery Strategy for the Atlantic whitefish (DFO 2006) that has the goal:

> *"to achieve stability in the current population of Atlantic whitefish in Nova Scotia, re-establishment of the anadromous form, and expansion beyond its current range."*

This goal is to be met through the implementation of four inter-related strategic objectives:

1. *Conserve, protect, and manage the species and its habitat;*
2. *Increase the number and range of viable populations;*
3. *Increase understanding of the species and its habitat, and;*
4. *Increase public involvement and acceptance.*

(DFO 2006)

Although recovery is considered to be feasible (DFO 2006), the design and implementation of specific recovery actions for Atlantic whitefish have been hampered by a lack of information on the biology, physiology, life history and habitat requirements. The success of activities implemented to ensure both the survival of the existing population and the recovery of the species via range extension are thought to depend on the adaptability of the remaining population to ongoing environmental and human-induced changes within the Petite Rivière lakes (survival) (Bradford et al. 2004b; DFO 2004) and the viability of the species to introduction into new freshwater and marine habitats (recovery) (DFO 2004).

In order to acquire information to help guide recovery activities, DFO in partnership with the Department of Biology, Dalhousie University, initiated a series of controlled experiments to assess the response of Atlantic whitefish eggs, larvae and juveniles to water pH, temperature, and salinity with survival and growth as the metrics of response. This document describes the experiments and reports the outcomes. The results are then applied to rivers with available water chemistry data within the Nova Scotia Southern Upland (SU) region lying within the

historical range of the species to assess recovery feasibility under a series of life history scenarios.

Regional Water Quality

The watersheds of the SU rivers are some of the most acidified in the province as they are generally characterized by shallow soils on top of igneous and metamorphic rocks lacking in basic minerals (Watt 1986). Although, paleolimnological reports show that many of these watersheds possess naturally low pHs (Ginn et al. 2007), the effect of acid rain from industrial pollution drove pHs downward to historic lows in the late 1970's (Clair et al. 2007). Since that time, pH has remained relatively stable and, with the subsequent decreases in sulphur emissions, model projections indicate pH will increase over the next several decades (Clair et al. 2004).

Within the SU, there is both spatial and seasonal variability in pH. Spatial variability occurs as intermittent geological deposits from the last glacial retreat (i.e., drumlin fields) offer a degree of buffering capacity to some rivers. Seasonally, pH is at its minimal level through winter, dipping slightly during the spring melt, rising in spring and summer to its maximum and then decreasing again with autumn freshet events (Watt et al. 1983).

Based on the observed spatial variability in pH, Watt (1986) proposed a categorization of SU rivers relating pH to the status of their Atlantic salmon stocks (Table 1). Although Atlantic whitefish and Atlantic salmon differ in biology and physiology, the same pH categories were employed in this study as they suggest significant changes in species assemblages; moreover, it is convenient to help assess the usefulness of Atlantic salmon as a model species.

Biological Considerations

For this work, emphasis was focused on the early life stages of Atlantic whitefish; as is the case with other species, they are the most sensitive to environmental factors (von Westernhagen 1988; Keinanen et al. 1998). Prior to describing experiments and results, the biology and behaviours of Atlantic whitefish are briefly described so that results can be interpreted in context.

Atlantic whitefish spawning has never been observed in the wild. Historical data indicates that gravid anadromous Atlantic whitefish ascended the Tusket River during late September to November (Bradford et al. 2004a). Both wild-caught lake resident Atlantic whitefish and their progeny raised in captivity to maturity spawn from late November to early January (J. Whitelaw, Mersey Biodiversity Facility, Milton, Nova Scotia, personal communication). The characteristics of suitable spawning habitat are not known although it appears certain that the Petite Rivière population spawn in lakes, as is typical elsewhere for both lake whitefish (*Coregonus clupeaformis*) and cisco (*Coregonus artedii)* (Scott and Crossman 1979).

Under culture, fertilized eggs are demersal and slightly adhesive (Hasselman et al. 2007). Observed water-hardened egg diameters vary between 3 and 5 mm (mean ± standard deviation (sd) = 3.4 ± 0.09 mm, A.M. Cook, unpublished data; mean ± sd = 4.1 ± 0.89 mm, Hasselman et al. 2007). Larvae hatch after incubating 260 ± 5.5 degree days (calculated as days from fertilization to hatch × incubation temperature in °C), which corresponds to an April-May hatch period in most natural environments in Nova Scotia (A.M. Cook, unpublished data). Estimated lengths at hatch vary from 12.4 mm ± 0.9 mm (Hasselman et al. 2007) to 14.2 ± 3.4 mm (A.M. Cook, unpublished data). After hatch, Atlantic whitefish spend approximately 15% of the

time swimming off the bottom. The amount of time spent within the water column increases daily until the onset of feeding (4-days post hatch) when they swim constantly (A.M. Cook, unpublished data). Metamorphosis to juveniles occurs around 30-days post hatch (Hasselman et al. 2007) and at 31 to 49 mm total length (Hasselman et. al. 2007; A.M. Cook, unpublished data).

The habitat preferences of immature Atlantic whitefish are not well understood. No age 0^+ or 1^+ fish have been captured in either Millipsigate or Minamkeak lakes. A single specimen, suspected to be age 0^+, was captured with a beach seine along the shoreline of Hebb Lake within the Petite watershed during June 2002 (Hasselman et al. 2005). Additional immature specimens were captured in a 5 m deep floating trap net installed in Hebb Lake during the autumn of 2006 (Bradford et al. 2010). The marine habitat preferences of the anadromous Tusket River population were never reported.

Observations in the field and under captive rearing indicate that Atlantic whitefish mature at a fork length of approximately 200 mm and as early as age 2^+ years.

Selection of Environmental Variables

To aid in the recovery of Atlantic whitefish and to increase the understanding of the species' biology and physiology, the environmental variables salinity, pH and temperature were chosen for detailed study. Salinity was chosen because Atlantic whitefish are considered to be anadromous by nature, were known to inhabit coastal waters, and to make upriver 'spawning' migrations as adults, at least on the Tusket River during the autumn months; however, there is no information on the timing of down river migrations and resultant survival at any life stage. Water pH was selected for detailed study because it likely helped shape the past freshwater distribution of Atlantic whitefish within the SU, an area known to possess inherently strong spatial variability in pH, and it is also the area that represents the geographic focus of recovery activities (DFO 2006). Moreover, assessing the effect of pH gives the contemporary impact of water quality on survival of Atlantic whitefish. Temperature was chosen as it is among the most influential environmental factors affecting survival, growth and production of fish species (Brett 1979). In addition, temperature is known to influence the response of species to other environmental factors (A.M. Cook, unpublished manuscript) so the interaction of temperature and pH was examined.

Salinity

Knowledge of how salinity may affect both survival and growth is of particular importance to the task of developing viable anadromous runs from a donor stock that has probably been land-locked for at least a century (Bradford et al. 2010). While reported occurrences in tidal waters of strays from the Petite Rivière lakes (Edge and Gilhen 2001; Bradford et al. 2004a) indicate some capacity for salt tolerance, neither the ontogeny of salt tolerance nor the timing of first descent to tidal waters are known for Atlantic whitefish.

Salinity affects the survival of fish by its influence on the ion-balance systems. Most species of fish maintain internal ion-concentrations corresponding to about 10-12 ppt salinity (Boeuf and Payan 2001). Residency in freshwater (0 ppt) requires an active uptake of ions and removal of excess water, whereas residency in seawater (30 ppt) requires uptake of water and removal of excess ions to maintain homeostasis. Stenohaline species are not capable of making these transitions between environments, whereas euryhaline species readily move between environments and alter their ion-regulating systems accordingly. Still other species are stenohaline at one life stage and euryhaline at another; Atlantic salmon is one example, as they

must undergo smoltification prior to transition into seawater. Salinity levels may also affect the growth of euryhaline species as there is a significant energetic cost associated with ion regulation.

Empirical determination of both the onset (ontogeny) of salt tolerance and the degree of salt tolerance possessed by Atlantic whitefish at discrete life history stages is necessary because the information drawn across the coregonid genus indicates substantive inter-specific variability. Lake whitefish (*C. clupeaformis*) are usually freshwater resident, although occasional forays into coastal habitats are known, and there are some indications that wild populations can breed and survive in 15 ppt (Rawson and Moore 1944). The European whitefish (*C. lavaretus*) exhibit wide diversity in their areas of occupancy relative to salinity across their natural geographic range. Some populations live exclusively in freshwater while others show preference to 20 ppt salinity during the early larval stages (Girsa et al. 1980). In contrast, two-week-old pelyad (*C. peled*) had 100% mortality at salinities above 8 ppt (Nesterenko 1976).

It is anticipated that knowledge of the effect of salinity on survival and growth of Atlantic whitefish life history stages can help establish the potential timing and duration of out-migration as well as the potential conservation benefit of recovery actions aimed at establishing anadromy among the Atlantic whitefish.

pH

The Tusket River in Nova Scotia's SU, which was known to support an Atlantic whitefish population at one time, was naturally acidic before the era of acute pH depression arising from acid rain (Clair et al. 2007; Ginn et al. 2007). The three Petite Rivière Lakes still possessing Atlantic whitefish have consistently maintained a pH greater than 5.6 (Ginn et al. 2008). Presently, pronounced differences in mean annual pH among SU rivers persist (Watt 1986), which may have implications for range extension and restocking.

Low pH can disrupt ion regulation below some threshold that tends to be both species and life stage specific. In freshwater, the fish's body fluids are hypertonic to their environment, which results in a net loss of ions and uptake of water. These factors are mitigated by chloride cells on the gills, which actively uptake environmental sodium (Na^{+}) and chloride (Cl). When water pH is reduced below the lower threshold, active uptake is substantially decreased causing an increase in the passive efflux of both ions through displacement of calcium (Ca^{+2}) ions with hydrogen (H^{+}) ions on the binding sites of the gill epithelium. This loss of ions results in a shift of water from extracellular fluids (i.e., blood) to intracellular fluids, which results in reduced blood volume and enlarged red blood cells that, in combination, increases viscosity and arterial pressure. Death may result from circulatory failure. The effects of pH are life stage and body size dependent. Embryos and early life stage fish generally do not have fully competent ion regulating systems and proportionally larger gill surface areas, both of which lead to a faster efflux of ions and increased susceptibility to acidified water (Schofield 1976; Fu et al. 2010). Sublethal effects of low pH include decreased growth rates as a result of increased standard metabolism, decreased appetite, and decreased food conversion efficiency (Rosseland and Skogheim 1987).

Available literature with regard to the effects of pH on the genus *Coregonus* causes concern for species/populations located in the Scandinavian countries where the effect of low pH is confounded by high (toxic) levels of aluminum (Keinanen et al. 1998; 2004). These studies are, therefore, of little value to the present assessment of pH effects on Atlantic whitefish because aluminum toxicity is not a factor in acidified Nova Scotia rivers. Briefly, the aluminum mobilized

at low pH in Nova Scotia's SU rivers becomes chelated by the high, natural total organic carbon load in river water (Lacroix and Townsend 1987).

Temperature

Temperature is generally regarded as the most important rate limiting environmental factor for fish, as its influence on bioenergetics will alter specific habitat's suitability for survival and growth (Brett 1979). Although temperature related responses are species and life history specific, most responses are generally nonlinear (Björnsson and Steinarsson 2002; A.M. Cook, unpublished manuscript), and exhibit an optima and upper and lower threshold bounds that can be fitted to a model of the form proposed by Parker (1974; Equation 1, Figure 2). Consideration of the full thermal physiology of fishes yields potentially valuable insight into optimum temperature for growth, maximum temperature where growth stops, the scope for growth and resistance to temperature change.

Interactions

The interaction of temperature and pH was also examined. Interactions are important to study as environments rarely change one factor at a time. Understanding the effects of pH and temperature interactions on Atlantic whitefish will allow for an increased predictive ability on habitat influences.

METHODS

Lab Based Experiments

Source of Test Animals

Atlantic whitefish used in these experiments were the F1 progeny of wild captured individuals, which had spawned successfully in captivity on at least one previous season. Spawnings were performed through dry fertilization at the Mersey Biodiversity Facility. Resultant progeny were transferred to Dalhousie University's Aquatron Laboratory as water hardened eggs, eyed eggs, yolk sac larvae and early feeding larvae.

Dalhousie Experimental Facility

The experimental lab located in the Dalhousie University Aquatron facility receives dechlorinated fresh water from the Municipality of Halifax's water supply, which has Pockwock Lake as a source. Sand-filtered sea water is supplied from the Northwest Arm of the Halifax Harbour. The lab consisted of 15 140 L rearing tanks, and five 60 L header tanks each feeding three experimental tanks. Each header tank can be controlled for either pH or salinity, with pH maintained using dosing pumps connected to multi-channel electronic temperature and pH controllers and salinity is adjusted by the mixing of salt and fresh water. Temperature is controlled in the experimental tanks through a combination of immersion heaters and by mixing heated and ambient freshwater. Treatment pHs in all experiments were achieved by raising or lowering the pH from acclimation levels at a maximum rate of 1.0 pH units per day.

As a method to calibrate the lab, and to ensure the portability of the results for Atlantic whitefish, an experiment was performed in the lab to compare the effect of low pH on Atlantic salmon alevins (see Fraser et al. 2008 for details) with some previously published relationships, developed from *in situ* experiments with young Atlantic salmon (Korman et al. 1994). Results

shown in Appendix 3 indicated that the Atlantic salmon survival curves generated under the controlled conditions of the Aquatron are congruent with those generated from wild populations.

Salinity

Tolerance

Salinity tolerance of Atlantic whitefish was assessed for larvae, juveniles and adult life stages. Individuals held in freshwater were acclimated to either 15 ppt or full strength seawater (30 ppt) at a rate of 5 ppt×0.5 day^{-1}. Salinity treatments were replicated twice for the larval and juvenile experiments with 50 and 20 individuals used within each replicate respectively. Adults were only tested at 0 and 30 ppt with one replicate consisting of 30 and 50 individuals, respectively. Results are shown as mean survival rates for each life stage tested and duration of the test for all life stages.

Preference

Salinity preference was assessed using juvenile Atlantic whitefish. Groups of 30 fish were acclimated to fresh (0 ppt), brackish (15 ppt) and full strength seawater (30 ppt) for two weeks prior to experiments. In each experiment, the three salinity preference chambers were filled with brackish, full strength seawater or freshwater. The freshwater chamber was allowed to overflow to set up a stratified layer of freshwater across the surface to allow for fish movement between chambers. Once filled, five fish from each acclimation salinity were introduced into each salinity chamber. Fish movements were monitored over 24-hours. Experiments were performed twice for each acclimation salinity.

pH

Fertilization Success

Eggs from two female Atlantic whitefish were dry fertilized with the milt of two Atlantic whitefish males at the Mersey Biodiversity Facility. Eggs and milt were allowed to mix for 60 seconds prior to division into two equal sized batches that were rinsed and water hardened for 60 minutes in freshwater of either pH 5 or 7. After the initial water-hardening, eggs were transferred to the experimental lab. Eggs from both pH batches were equally divided into triplicate groups of 35 individuals destined for each of the treatment pHs set to 4.1, 4.3, 4.5, 5.0 and 7.0 where they remained for two weeks at temperatures between 4.6-6.1°C. Mortalities were tallied and removed as observed. At the end of two weeks, the numbers of viable eggs per treatment were counted.

Egg Viability

Eggs from five discreet spawnings (family groups) incubated to the eyed stage at the Mersey Biodiversity Facility were moved to the experimental lab where each family group was divided into 15 batches of 20 individuals. Batches were moved to one of five pH treatment tanks, which were initially set to pH 5.1 (equivalent to the Mersey Biodiversity Facility at time of transfer). Treatment pHs of 4.0, 4.3, 4.6, 5.2 and 7.3 were achieved by changing the pH by a maximum rate of 1.0 pH unit per day. Eggs were checked daily, mortalities were counted and removed as observed. pH and temperature were measured twice daily, until the termination of the experiment, eight days after the 50% hatch date.

Survival of Larvae and Juveniles

Three experiments were conducted to assess the effect of low pH in late stage larvae, early juvenile, and juvenile. The first experiment, using late stage larvae with a mean initial body size of 24 ± 0.3 mm, assessed survival at treatment pHs of 3.9, 4.1, 4.3, 5.0 and 7.0 at a common temperature of 14.0°C. Twenty individuals were randomly allocated to triplicated treatments. Fish were fed four to five times daily, with mortalities tallied and removed as observed. Survival was assessed at the end of the eight day experiment.

The second experiment used juvenile Atlantic whitefish at a fork length of 39 ± 3.3 mm. Treatment pHs were 4.0, 4.2, 5.0 and 7.0 at a common temperature of 18.0°C. Forty fish were randomly allocated to triplicate treatments. Fish were fed two to three times daily with mortalities removed as observed. Survival was assessed at the end of the 15 day experiment.

The third experiment used juvenile Atlantic whitefish (initial body size of 69 ±8.0 mm). Treatment pHs were 4.0, 4.2, 4.7, 5.0 and 7.0 at a temperature of 20.0°C. Fifty fish were randomly allocated to triplicate treatments. Fish were fed twice daily and mortalities were recorded as observed. Survival rates were calculated at the end of the 16 day experiment.

Interactions

Growth ~ Temperature × pH

The effects of temperature and pH on growth of juvenile Atlantic whitefish were examined with a 4×7 factorial experiment. Temperatures and pH combinations were 3.1°C, 4.5°C, 10.5°C, 14.2°C, 17.0°C, 20.0°C or 22.4°C and 4.0, 4.2, 4.75 or 7.2, respectively. Tanks of 20 fish ranging in body size from 60-100 mm were reared at treatment levels for 14 days. Fish were hand fed two to three times daily for the duration of the experiment. All fish were measured to fork length (nearest 1 mm) at the start and completion of the experiment in order to estimate growth rates. Growth rates (mm · d^{-1}) from each pH treatment (four models) were fitted to the empirical growth-temperature model (Equation 1; Parker 1974) using nonlinear least squares estimation (nls package in R).

$$G = G_{\max}\left[\left(\frac{T}{T_{opt}}\right)\times\left(\frac{T_{\max}-T}{T_{\max}-T_{opt}}\right)^{\left(\frac{T_{\max}-T_{opt}}{T_{opt}}\right)}\right]^{\alpha} \qquad \text{Eq. 1}$$

where G is growth (mm · d^{-1}), G_{max} is the maximum observed growth rate, T is temperature in °C, T_{opt} is the temperature in °C where growth is maximized, T_{max} is the high temperature in °C where growth drops to zero and α is the shape parameter. The four models were compared by the addition of indicator variables (X) and incremental parameters (Δ's) into Parker's original model (see Cook et al. 2006) yielding:

$$G = \left(G_{\max}-\Delta G_{\max}\cdot X\right)\left[\left(\frac{T}{T_{opt}-\Delta T_{opt}\cdot X}\right)\times\left(\frac{\left(T_{\max}-\Delta T_{\max}\cdot X\right)-T}{\left(T_{\max}-\Delta T_{\max}\cdot X\right)-\left(T_{opt}-\Delta T_{opt}\cdot X\right)}\right)^{\left(\frac{\left(T_{\max}-\Delta T_{\max}\cdot X\right)-\left(T_{opt}-\Delta T_{opt}\cdot X\right)}{\left(T_{opt}-\Delta T_{opt}\cdot X\right)}\right)}\right]^{\left(\alpha-\Delta\alpha\cdot X\right)}$$

Scope for growth was defined as the area under the response curve for each treatment pH (Figure 2). Thermal resistance, defined as the area under the response curve between the optimum to maximum temperature (Figure 2), was calculated for each pH treatment. Comparison of area among pH treatments was facilitated via standardization of models.

The calculated range of optimum temperatures, maximum temperatures, scopes for growth and thermal resistances for Atlantic whitefish were plotted along with those from other salmonids to investigate interspecific variability.

Survival Models

Physiologically-based life stage specific survival curves (PBLS) were generated to depict the effects of pH and salinity on the early life stages of Atlantic whitefish. Models were fit by nonlinear least squares estimation (nls package in R), using the data from experiments concerning the effect of pH on 1) egg-incubation, 2) egg-hatching, 3) larval survival, 4) early juvenile and 5) juvenile survival. In addition, the effect of salinity on larval survival was modeled. The effect of salinity on other life stages was not included as fish spawn and eggs are incubated in freshwater and all later stages (juvenile onward) exhibited 100% tolerance in controlled experiments. All PBLS models were of the form:

$$A+(R-A)e^{-t\cdot V} \qquad \text{Eq. 3}$$

where *A* is the asymptote, *R* is the y-intercept, *t* is the rate of change and V is the experimental variable, either pH or salinity.

River Data

Time series of pH data was obtained for 18 rivers from the SU region of Nova Scotia. These data were collected over a 25 year period (1979 - early 2000's) at irregular intervals and variable frequency among rivers by a variety of groups including DFO, Environment Canada, Nova Scotia Department of Fisheries and Aquaculture and Nova Scotia Power Inc. While the river specific data sets were not equivalent in size or number of sample sites, all represent time series that covered at least 10 months of the year (Appendix 1). Monthly mean and standard deviation of pH levels were calculated for each river (Appendix 1; Feinstein 1979). Missing months were estimated as the intermediate value of the adjacent months. Rivers were categorized into groups according to the criteria developed by Watt (1986) for Atlantic salmon.

Model Simulations

River specific survival probabilities were generated by coupling the PBLS curves with the monthly pH information. Atlantic whitefish spawning time and developmental rates were assumed to be the same for all rivers with egg incubation occurring between December and March, hatching occurring in April, larval stages in May and early juvenile and juvenile stages in June and from July to December, respectively.

Simulated river specific survival probabilities for Atlantic whitefish were generated from fertilization to the end of the first year of life following different life history scenarios (see below). Within a river, monthly pH data were sampled from a normal distribution constrained by the observed mean and standard deviations (Appendix 1). The PBLS models were used to generate survival estimates by month that, in turn, were used to estimate cumulative survival

over the annual time series. This procedure was performed 1000 times for each river in order to generate the distribution of survival probabilities.

Life History Tactics

The effect of complete freshwater residency versus anadromy on the distribution of survival probabilities was explored for three scenarios using model simulations. The first scenario assumed freshwater residency from spawning to the end of December of the first year. The second scenario assumed seaward migration just after hatch, which leads to the incorporation of the PBLS model for larval survival in salt water, and then 100% survival of juveniles onward to end of December, following results from experimental data. The third scenario assumed seaward migration as early (post-metamorphosis) juveniles, i.e., when fully saltwater tolerant but must survive the ambient pH of the river for the month of May. In all three scenarios, simulations began at fertilization in December and continued for 12 months until the following December. The distributions of survival probabilities for each scenario and river category were compared using Kolmogorov-Smirnov tests.

RESULTS

Lab Based Experiments

Salinity

Salinity tolerance of Atlantic whitefish was life stage dependent. Larval survival decreased moderately from 100% in freshwater to 94% and 92% at 15 ppt and 30 ppt, respectively. Juveniles and adults were fully tolerant of full seawater (30 ppt; Table 2; adult mortalities were caused by other non-experimental factors). The ontogenetic increase in salinity tolerance is mirrored by the preference for marine salinity levels in juveniles. Experimental results show that regardless of acclimation salinity, juveniles almost exclusively (mean $\pm$ sd = 92.2 $\pm$ 5.0%) prefer full strength seawater (30 ppt; Figure 3) but only following an acclimatization period of 3-10 hours depending upon the rearing salinity (i.e., fresh (0 ppt), brackish (15 ppt); Figure 3, upper, middle).

pH

Low pHs decreased the survival of Atlantic whitefish for all life history stages tested (Table 3; Figure 4). Generally pHs less than 5.0 decreased survival in eggs, whereas pHs less than 4.5 decreased survival of larval and juvenile Atlantic whitefish (Figure 4). The most to least sensitive stages are egg>hatch>larvae=early juveniles>juveniles. PBLS models described the relationship between pH and survival across all life stages (Figure 4). However, the PBLS models for the egg and larval stages underestimate survival rates due to the substantial observed variability. The generated estimates of survival are, therefore, considered to be conservative (Figure 4).

Temperature

The thermal physiology of juvenile Atlantic whitefish was described by having an optimum growth temperature of 16.5°C, maximum growth temperature (high temperature representing zero growth) of 24.6°C, a scope for growth of 11.7 and a thermal resistance of 4.9 (results from pH 7.2; Table 4; Figure 5).

Comparing the thermal physiology of Atlantic whitefish to Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*) and sockeye salmon (*Oncorhynchus nerka*) suggests that they have an intermediate optimum temperature most similar to sockeye (16.4°C). They are, however, more similar in thermal physiology to Atlantic salmon both of which possess the highest levels of maximum growth temperature, scope for growth and thermal resistance (Figure 6).

Interactions

Temperature × low pH interactions were examined across the full range of levels Atlantic whitefish would experience in the SU region of Nova Scotia. Differences in the thermal physiological profiles estimated for pHs 4.75 and 7.2 are small and not significant. Moreover, optimum temperature for growth did not change significantly with pHs from 4.0 to 7.2 with an overall range of 15.5°C -16.5°C. However, both maximum growth (*Gmax*), and maximum growth temperature decreased significantly at pHs < 4.75 (Figure 5). Specifically maximum growth and maximum growth temperature decreased by approximately 1.0 mm · d^{-1} and 3.8°C, respectively, as pH decreased from 7.2 to 4.0 (Table 4).

Thermal sensitivity increased with decreased pH as both scope for growth and thermal resistance decreased linearly from 7.2 to 4.2 (Figure 7). Further decreases in pH from 4.2 to 4.0 results in a significant decline in both suggesting a rapid decrease in ability to tolerate temperature changes (Figure 7).

Model Simulations

Survival probabilities (mean ± sd = 0.85 ± 0.06) were significantly higher in moderate (pH Category 3) to neutral (pH Category 4) rivers than Category 1 and 2 (mean ± sd 0.49 ± 0.15) rivers regardless of life history strategy (Table 5; Figure 8). The greatest difference between adjacent categories was for Category 2 versus 3 where the increase in pH for Category 3 rivers yielded an improvement in survival probability of 0.28 (Table 5).

The advantage of anadromy over freshwater residency for Atlantic whitefish was most evident in the lowest pH rivers (Category 1) as there is a relaxation of pH induced mortality raising the survival probability by approximately 30%, regardless of stage of migration (Table 5, Figure 9). Similarly, Atlantic whitefish in Category 2 rivers could be expected to increase their survival by approximately 10% to 15% by migrating to sea as larvae and juveniles, respectively. Larval anadromy decreased survival probabilities in both Category 3 and 4 rivers by means ± sd of 1.45 ± 1.66% and 4.35 ± 0.65% because of the increased physiological mortality associated with seawater (Figure 4; 8; 9). In contrast, prolonged freshwater residency and juvenile anadromous migrations from Category 3 or 4 rivers will increase the survival rates albeit by marginal levels (<5%; Figure 8; 9).

Variability in survival probability was highest in Category 2 rivers where pH regimes fluctuate near the Atlantic whitefish's tolerable range (Figure 6, Appendix 1), suggesting a recategorization of rivers may be necessary to meet the habitat requirements of Atlantic whitefish.

DISCUSSION

The decreased survival of Atlantic whitefish at pH levels representative of those expected prior to the era of anthropogenic river acidification suggests that it may have played a role in the demographics of the species: a naturally restricted geographic range. That being said, the relative importance of pH will likely never be known because: 1) there are many rivers in the area that are predicted to possess suitable pH profiles but were never known to have either freshwater or anadromous populations of Atlantic whitefish; 2) there are no records of Atlantic whitefish occurrences prior to the era of extensive dam construction on rivers within the SU region of Nova Scotia (Bradford et al. 2010); and 3) the introduction of non-indigenous predators and habitat destruction (Bradford et al. 2004b).

Compared to conspecific species, Atlantic whitefish possess an intermediate level of pH tolerance. Atlantic whitefish are more tolerant than Atlantic salmon across all life stages (summary in Korman et al. 1994 and Farmer 2000) but less tolerant than American eel (*Anguilla rostrata;* K.C. Reynolds, Dalhousie University, Halifax, NS, personal communication) and potentially less tolerant than either brook trout (*Salvelinus fontinalis*) or yellow perch (*Perca flavescens*) (Smith et al. 1986). Atlantic whitefish show increased tolerance to low pH from eggs through to juvenile stages. Similar increases in tolerance to low pH have been observed in most other species studied (Keinanen et al. 1998, 2004), although Atlantic salmon exhibit increases in tolerance from egg to parr but decreases in tolerance during the intermediate fry stage (Korman et al. 1994; Farmer 2000). Generally increased tolerance with stage or size is related to the improved ion regulating ability and/or decreased gill surface area to body size ratio as mentioned above and as is likely the case for Atlantic whitefish.

Atlantic whitefish can potentially initiate out-migration early in their first year, as they tolerate seawater as larvae and are fully seawater tolerant as early juveniles. Whether early out-migration was either a strategy adopted by the historical wild anadromous Atlantic whitefish or could be a successful life history tactic cannot, however, be known with confidence until *in situ* experiments in establishing anadromy are executed. However, the fact that out-migration of Atlantic whitefish as larvae (approximately 14 mm at hatch) or juveniles (5-10 cm) would not have been easily detected, particularly during early spring, from casual observation suggests that absence of historical information on the onset of out-migration cannot be considered as an indication that it did not occur. Further, the observed active swimming in newly hatched larvae and the evident preference of juveniles for seawater over fresh or brackish water are reasons for optimism that anadromy remains possible. Support of the "early out-migration" hypothesis can be found in another coregonid (*Coregonus lavaretus*) in the Gulf of Bothnia, which are known to migrate from their freshwater spawning areas to the marine environment during the spring-early summer (Lehtonen et al. 1992).

The thermal physiology (TPP) of Atlantic whitefish is similar to other salmoniformes, which are generally some of the most thermally sensitive species (A.M. Cook, unpublished manuscript). Specifically, Atlantic whitefish have a similar optimum temperature and scope for growth, but are among the highest examined for maximum growth temperature and thermal resistance. Observed species differences in TPP may be partially described by specific habitat choices and range of distribution in freshwater. The lower maximum growth temperature and lower thermal resistance for both Arctic charr and sockeye salmon, for example, likely result from their more northerly distribution and lake residency, respectively. Comparatively, the streams and rivers inhabited by young Atlantic salmon offer limited thermal refugia, thereby increasing their TPP.

Atlantic whitefish have optimum temperatures similar to the cooler water species, but their maximum temperatures and thermal resistances are closer to Atlantic salmon. Habitat

implications of Atlantic whitefish's TPP suggest that the lower optimum may be indicative of either lake residency or further evidence for an early out-migration to cooler estuaries (Figure 6). The higher maximum growth temperature and thermal resistance may indicate stream residency or perhaps is an adaptive trait acquired, as Atlantic whitefish have been constrained within the three semi natural shallow lakes for perhaps the last century. It is important to note that this comparison is only within salmoniformes and does not show their comparatively low TPPs in relation to other freshwater groups (A.M. Cook, unpublished manuscript).

Of particular interest is the change in thermal physiology with low pH. For the first time, the interaction of low pH and temperature was described across a species entire physiological range. Results showed that the growth of Atlantic whitefish was affected by the interaction of temperature and pH. At pHs below 4.75, the maximum temperature and growth rate significantly decreased ($p<0.05$), whereas the optimum temperature did not. Decreased maximum growth with declining pH was most likely due to the increased metabolic demand of ion regulation as well as decreased food intake (data not shown; Wood and McDonald 1982). Decreased maximum temperature with decreasing pH was likely driven by the increased blood viscosity associated with low pH coupled with the decreased cardiac output at high temperatures (Wood and McDonald 1982; Mark et al. 2002). The resultant decreased blood flow often results in death because of the fish's failure to meet metabolic oxygen demands (Pörtner et al. 2004). Similarly, studies on rainbow trout (*Oncorhynchus* mykiss) have shown that low pH and high temperature result in decreased resistance time (time to 50% mortality), which were attributed to the decreased time to respiratory failure from blood acidosis (Robinson et al. 1976).

Simulation Results and Atlantic Whitefish Introductions

Introduction of Atlantic whitefish can potentially be initiated in any category river in Nova Scotia's SU region, as survival is possible within any of the observed pH regimes. There are, however, advantages to stocking in either Category 3 or 4 rivers as survival, and therefore productivity, will be higher irrespective of life history strategy. In both Category 1 and 2 rivers, anadromous migrations significantly improve the survival probability, low pH rivers could, therefore, be considered as candidate stocking sites to establish anadromy. The difference in survival among pH categories can largely be explained by the long exposure to low pH during the sensitive egg stage. And as eggs are obligatory to freshwater, these pH effects cannot be directly mitigated through anadromy.

The thermal physiological profiles were not incorporated into the model simulations as complete temperature data were not available. In addition, there is very little information on the thermal habitat preferred by young Atlantic whitefish (Hasselman et al. 2005). However, it is important to be aware of the potential interactions between temperature and pH when assessing habitat suitability. The availability of thermal refugia in areas possessing low pH profiles (Category 1 or 2 rivers) should be examined prior to the stocking of Atlantic whitefish.

This work does not incorporate any other potentially confounding factors to species recovery such as land use practices, non-native predators or adequate fish passage. Prior to stocking, these factors need to be evaluated and considered in the context of the information presented here. As an example, Atlantic whitefish introductions may still proceed in watersheds with introduced predators if downstream migration of anadromous larvae is available, as spatial and temporal overlap between species may be negligible.

RESEARCH RECOMMENDATIONS

In this work, the feasibility of successfully introducing Atlantic whitefish in new areas to fulfill the strategic objective of the Recovery Strategy is demonstrated. Empirical determination of the results are recommended as a research priority. Further introductions should proceed using the best available information from the current work coupled with the Atlantic whitefish decision support tool.

A recategorization of rivers into potential Atlantic whitefish rivers should be performed. The original classification was based on the stock status of Atlantic salmon in each river, and it is not generally applicable as results have shown that Atlantic whitefish are more tolerant than Atlantic salmon to low pH, particularly across certain life stages (Appendix 3). This is particularly evident in the large variability in survival probability for Category 2 rivers. Defining a new classification scheme would provide further direction for the restocking and repatriation of Atlantic whitefish in the SU region of Nova Scotia.

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TABLES

Table 1: Categorization of Southern Upland rivers and the status of their Atlantic salmon stocks (after Watt 1986).

Category	pH	Atlantic salmon status
1	<4.7	Extinct
2	4.7-5.1	Remnant
3	5.1-5.4	Depleted
4	>5.4	Unaffected

Table 2: Survival rates of different life staged Atlantic whitefish exposed to freshwater (0 ppt), brackish (15 ppt) or full strength seawater (30 ppt).

Life stage	Body Size (mm)	n	Salinity (ppt)	Duration (day)	Survival (%)
Larvae	24	50	0	14	100
	24	50	15	14	94
	24	50	30	14	92
Juvenile	41	20	0	14	100
	41	20	15	14	100
	41	20	30	14	100
Adult	240-380	30	0	390	100
	220-430	50	30	390	96

*Table 3: Parameter estimates for the PBLS models used to predict survival of Atlantic whitefish life stages to pH or salinity. Significance p<0.05***.*

Stage	Parameter	Estimate	SE of Estimate	Significance level (*<0.05,**<0.01,***<0.001)	Residual SE
Eggs pH	A	0.98	0.03	***	0.152
	R	-2072	1746		
	t	0.65	0.11	***	
Hatch pH	A	1.03	0.06	***	0.151
	R	-7070	15050		
	t	0.82	0.24	**	
Larvae pH	A	1.03	0.06	***	0.122
	R	-566500	1686000		
	t	1.23	0.22	***	
Early juv. pH	A	1.00	0.05	***	0.09
	R	-7495000	25600000		
	t	1.38	0.21	***	
Juv. pH	A	1.00	0.01	***	0.02
	R	-533900000	2506000000		
	t	1.70	0.21	***	
Larvae salinity	A	0.906111	0.00776	***	0.004
	R	1	0.002236	***	
	t	-2.543676	0.222758	***	

*Table 4: Parameter estimates of the temperature growth model for Atlantic whitefish at four different pH levels. Significance p<0.05**, p<0.01***.*

pH	Parameter	Parameter Estimate	SE of estimate	Significance	Residual SE
4	G_{max}	1.7	0.023	***	0.03
	T_{opt}	15.5	0.150	***	
	T_{max}	20.8	0.228	***	
	α	3.1	0.236	**	
4.2	G_{max}	2.2	0.007	***	0.009
	T_{opt}	15.5	0.025	***	
	T_{max}	22.9	0.050	***	
	α	3.3	0.044	***	
4.75	G_{max}	2.7	0.020	***	0.026
	T_{opt}	16.3	0.064	***	
	T_{max}	24.2	0.217	***	
	α	3.6	0.137	***	
7.2	G_{max}	2.7	0.009	***	0.013
	T_{opt}	16.5	0.037	***	
	T_{max}	24.6	0.149	***	
	α	3.3	0.066	***	

Table 5: The effect of habitat choice in determining effects on relative survival of Atlantic whitefish through their first year of life.

pH Category	River	Freshwater	Larval anadromy	Juvenile anadromy
1 (<4.7)	Barrington	0.30	0.38	0.40
	Clyde	0.29	0.37	0.38
	Jordan	0.31	0.40	0.41
	Roseway	0.20	0.28	0.29
2 (4.7-5.1)	East	0.50	0.56	0.58
	Mersey	0.72	0.74	0.78
	Middle	0.59	0.61	0.64
	Sissiboo	0.63	0.66	0.69
	Tusket	0.49	0.55	0.57
	Cannan	0.44	0.51	0.53
	Salmon	0.38	0.47	0.48
3 (5.1-5.4)	Carleton	0.87	0.84	0.89
	Gold	0.75	0.75	0.79
	Lahave	0.88	0.85	0.89
	Medway	0.79	0.79	0.83
	Sackville	0.79	0.78	0.83
4 (>5.4)	Mushamush	0.96	0.91	0.96
	Petite	0.89	0.86	0.90

FIGURES

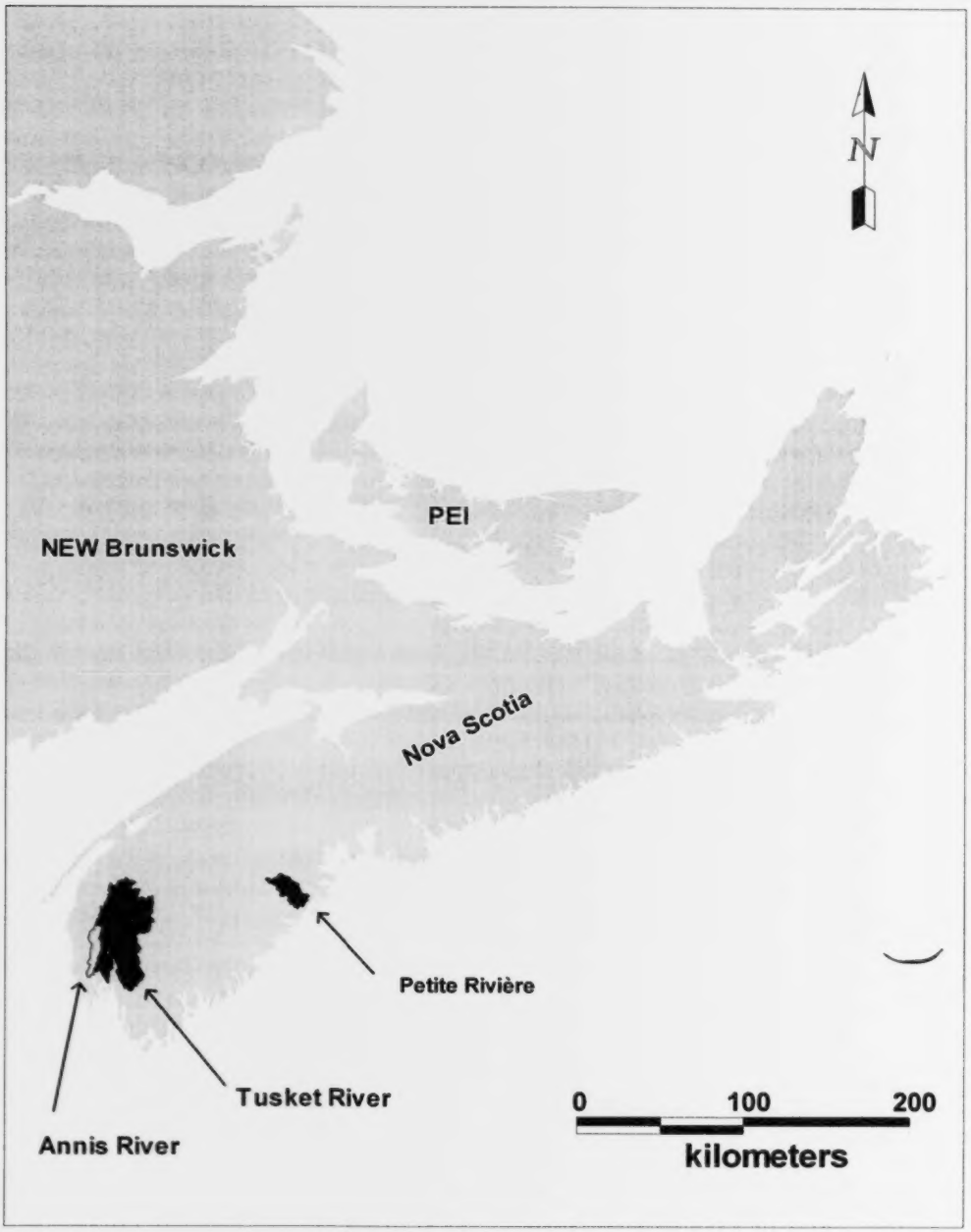


Figure 1: Location of the Petite Rivière, Tusket River and Annis River watersheds, Nova Scotia.

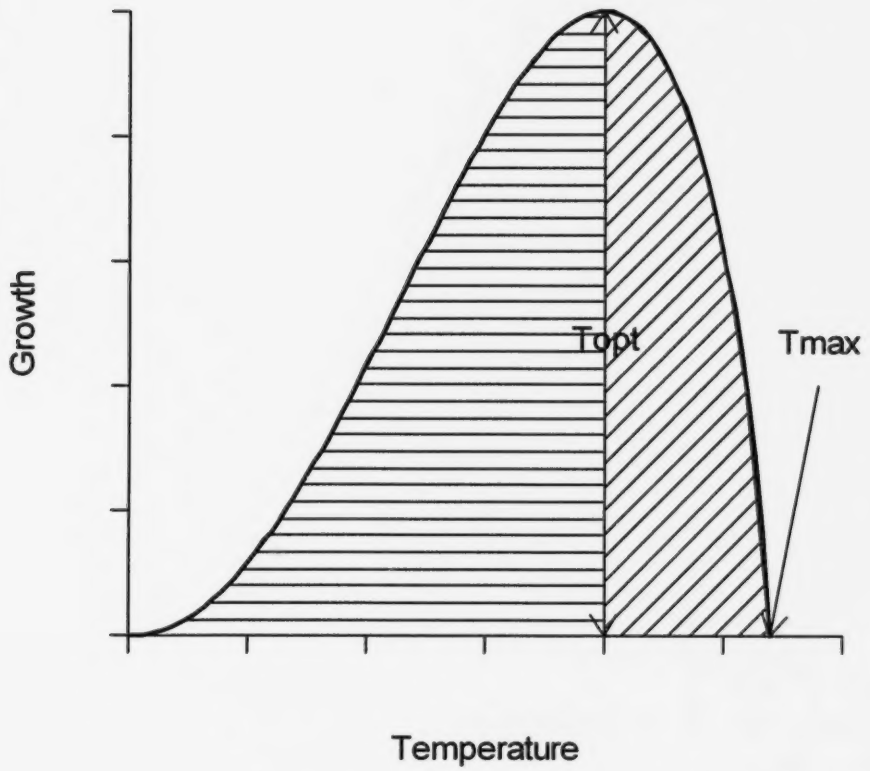


Figure 2: Temperature-growth relationship, where Topt is the temperature where growth is optimized, Tmax is the high temperature where growth is zero. The entire hatched area represents the scope for growth. The angled shading represents the thermal resistance.

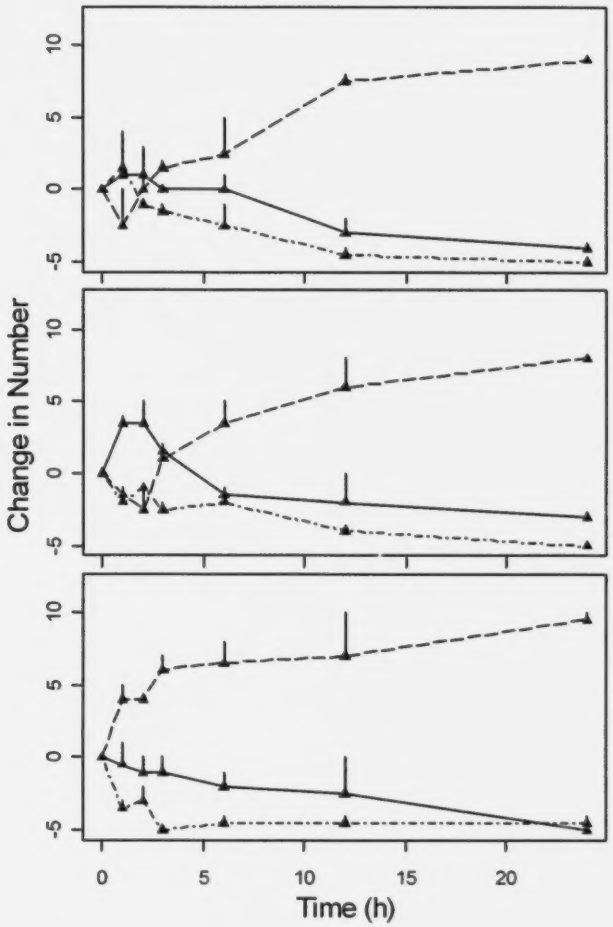


Figure 3: Salinity preference of juvenile Atlantic whitefish acclimated to 0 (upper), 15 (middle) and 30 (lower) parts per thousand (ppt) salinity. Change in number represents the gain or loss in fish over time (hours) from the original five fish placed in each salinity chamber (0-dash-dot, 15-solid and 30-dash ppt). Upper lines from symbols represent standard error.

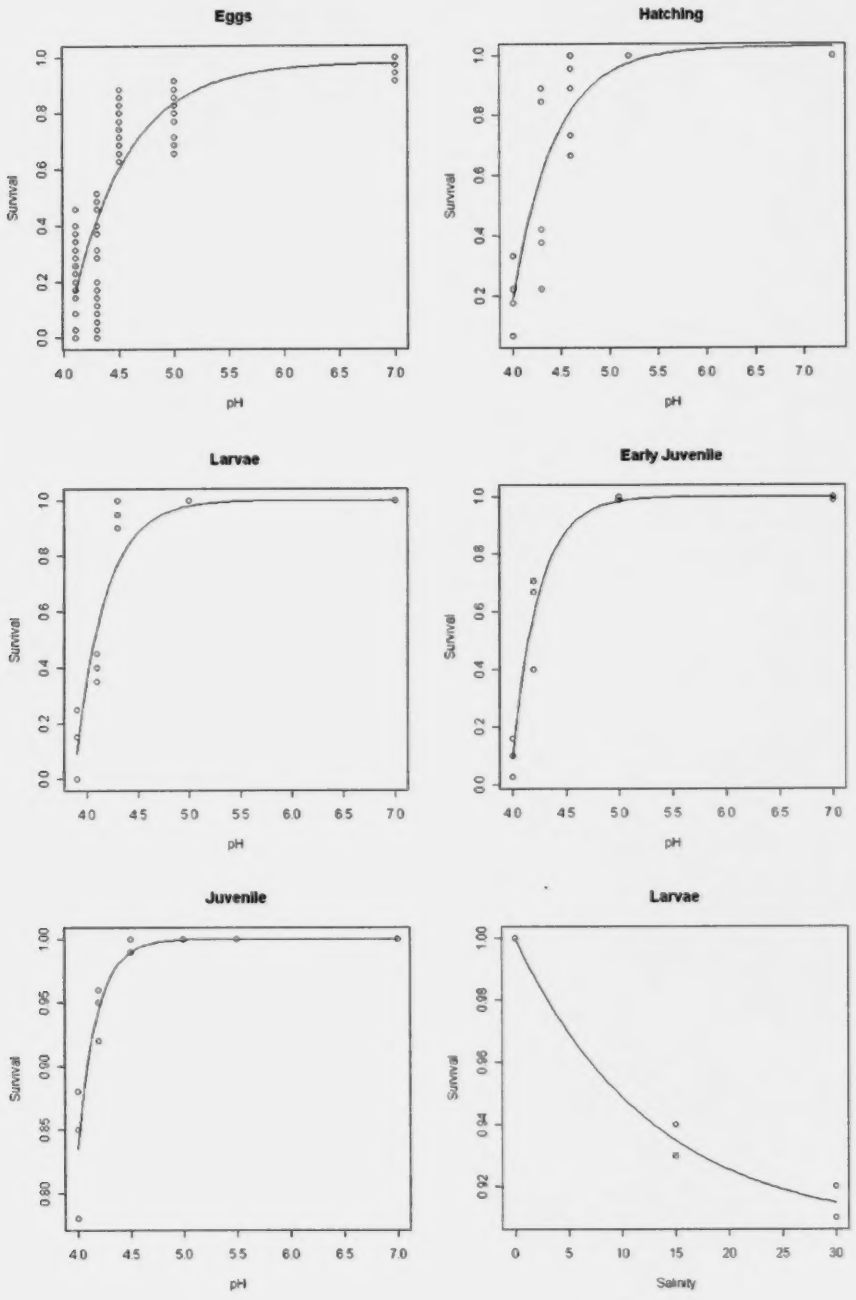


Figure 4: Survival rates of Atlantic whitefish life history stages as effected by pH or salinity. Regression fits are for the physiologically-based life stage specific survival curves (PBLS).

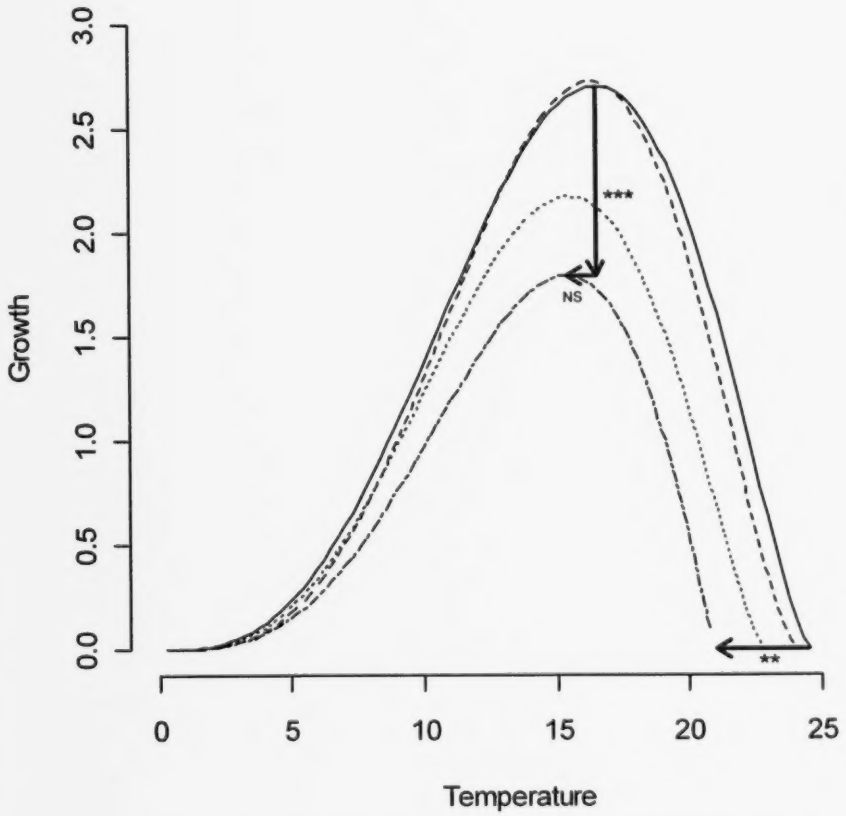


*Figure 5: Temperature-growth (mm · d^{-1}) relationships of Atlantic whitefish in pH levels of 7.2 (solid), 4.75 (dashed), 4.2 (dotted) and 4.0 (dashed-dotted). Solid arrows represent the direction of change with decreasing pH (significance denoted by NS ($p>0.05$), **($p<0.05$), ***($p<0.001$).*

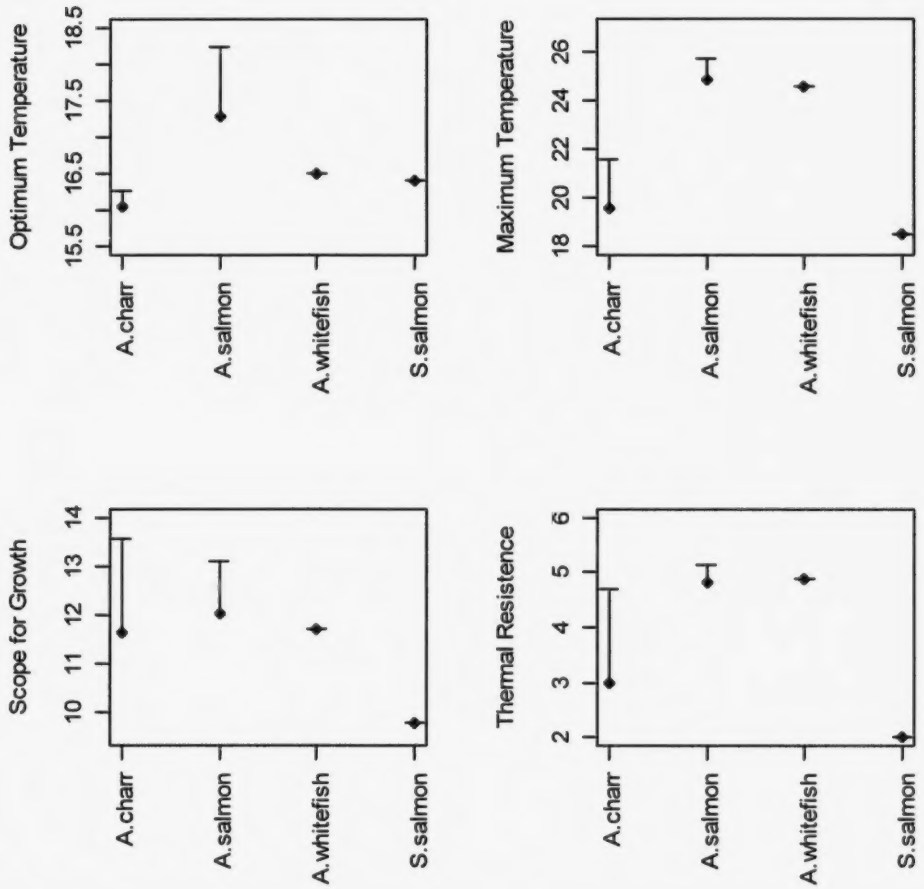


Figure 6: Comparison of the optimum temperature (°C), maximum temperature (°C), scope for growth and thermal resistance of several salmonid groups. Arctic charr (Salvelinus alpinus) and Atlantic salmon (Salmo salar) values are from two and four populations, respectively, Atlantic whitefish (Coregonus huntsmani) and sockeye salmon (Oncorhynchus nerka) are from single experiments. In all studies, fish were between 8 and 12 cm.

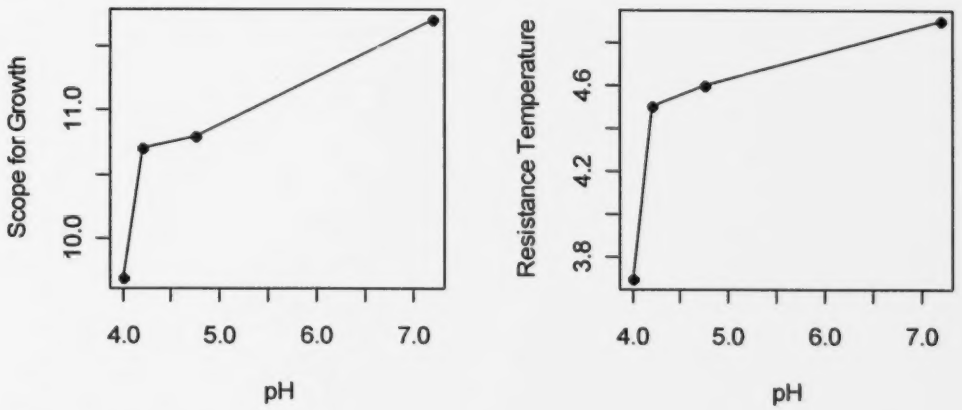


Figure 7: Changes in scope for growth and resistance temperature with pH for juvenile Atlantic whitefish.

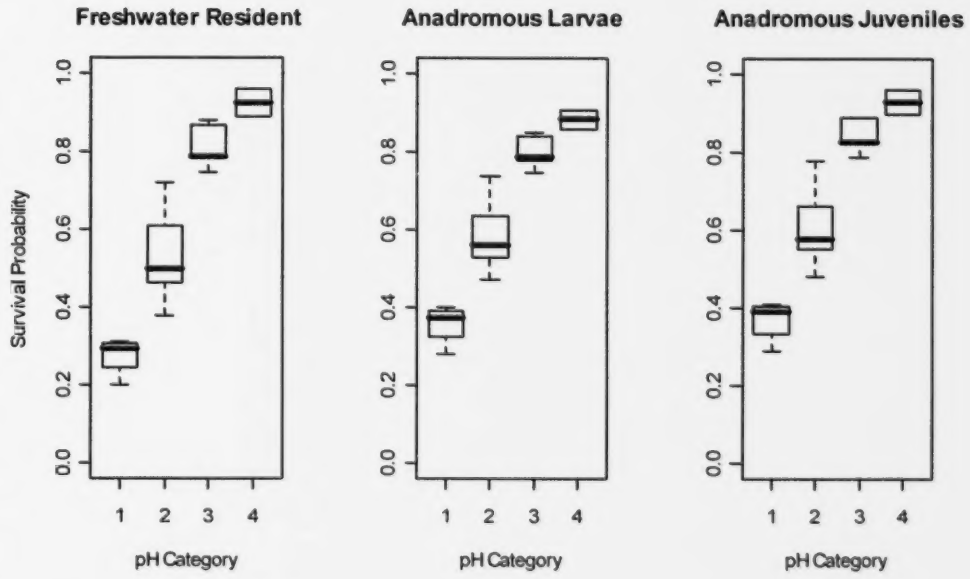


Figure 8: Survival probabilities of Atlantic whitefish across river pH categories as affected by different habitat choices.

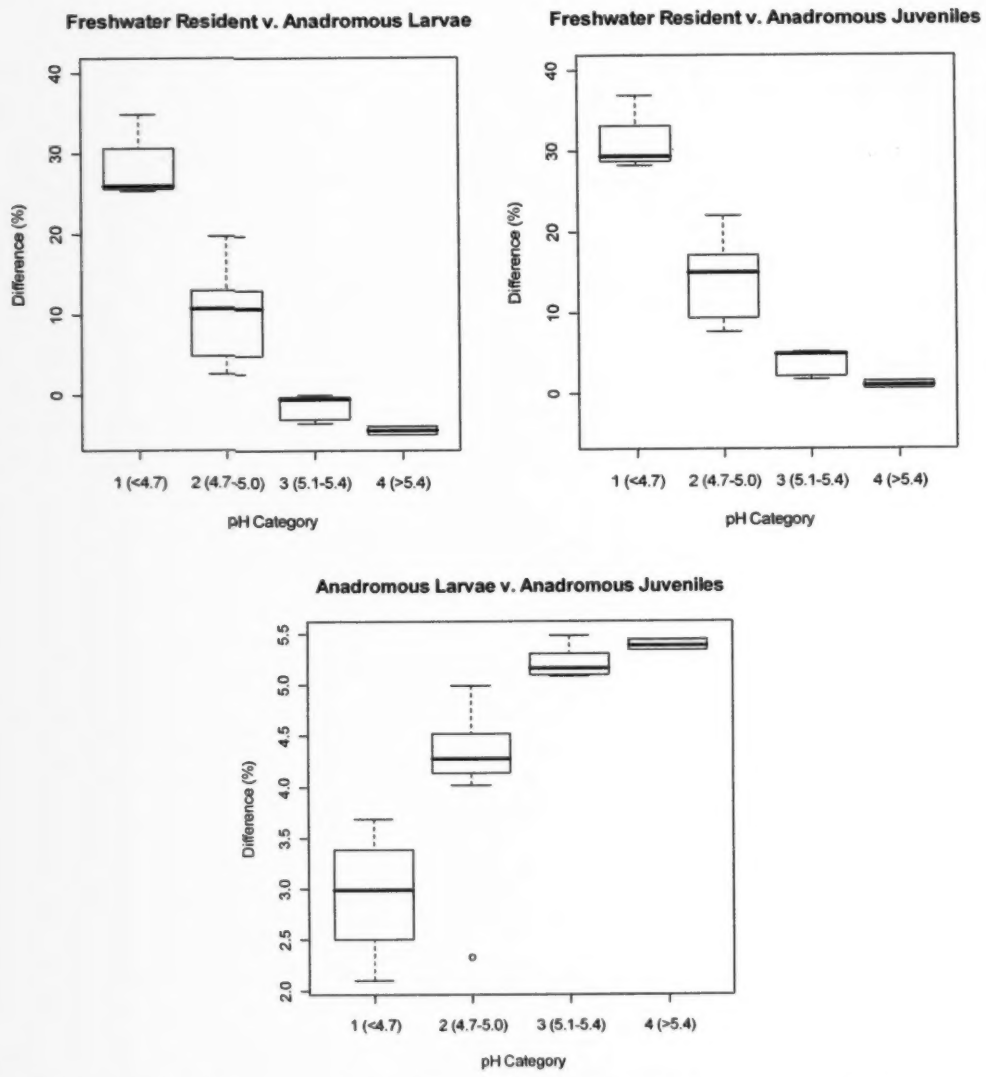


Figure 9: Difference (%) in survival probabilities for Atlantic whitefish that remain freshwater resident versus those which migrate to seawater during either larval or juvenile phases. Boxplots are grouped by pH category river, with Category 1 rivers representing low annual pHs (<4.7) through to Category 4 rivers which represent near neutral pH rivers (>5.4).

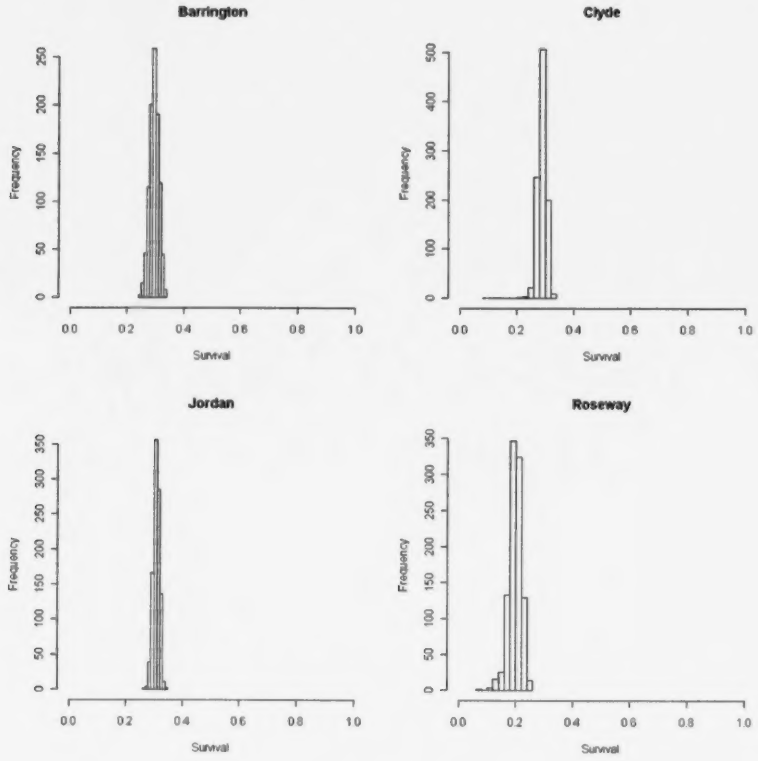
APPENDICES

Appendix 1: Mean and standard deviation of river specific pHs sampled on a monthly basis.

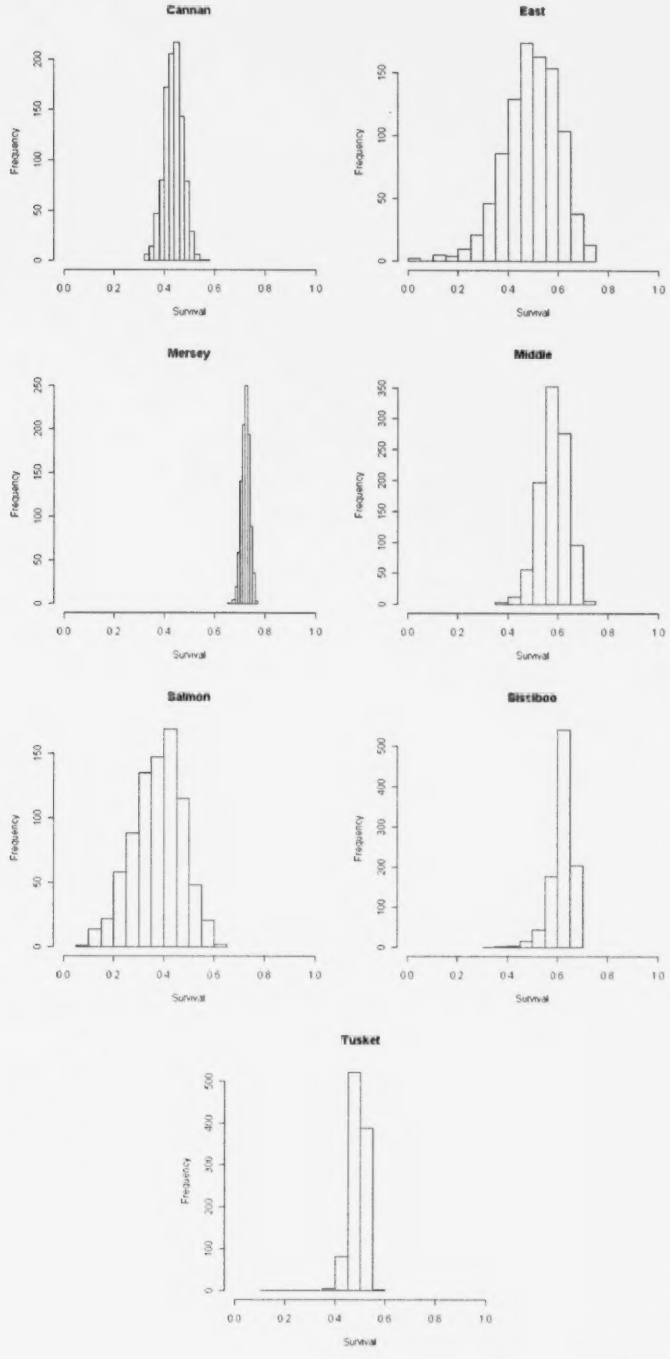
		River																	
		Barrington	Cannan	Carleton	Clyde	East	Gold	Jordan	Lahave	Medway	Mersey	Middle	Musharnush	Petite	Roseway	Sackville	Salmon	Sissiboo	Tusket
January	mean	4.53	4.61	5.45	4.46	4.75	5.06	4.39	5.49	5.1	5.07	4.78	6.33	5.63	4.25	5.17	4.63	4.74	4.58
	SD	1	1.026	1	1	1.071	1.058	1	1.036	1.009	1.026	1.044	1	1.059	1.001	1.065	1.123	1.029	1.01
February	mean	4.39	4.56	5.38	4.4	4.77	5.05	4.48	5.47	5.46	5.05	4.8	6.18	5.51	4.3	5.23	4.65	4.8	4.83
	SD	1	1.021	1	1	1.101	1.034	1	1.055	1.039	1.005	1.034	1	1.063	1.011	1.069	1.11	1.027	1.055
March	mean	4.45	4.56	5.58	4.48	4.74	5.09	4.52	5.52	5.26	4.98	4.79	6.15	5.53	4.57	5.46	4.57	4.74	4.66
	SD	1.009	1.022	1.008	1.006	1.05	1.049	1.007	1.048	1.064	1.022	1.049	1.016	1.066	1.052	1.065	1.043	1	1.006
April	mean	4.51	4.66	5.53	4.46	4.72	5.13	4.56	5.54	5.18	5.05	4.86	6.3	5.66	4.4	5.24	4.62	4.88	4.63
	SD	1.009	1.016	1.011	1.008	1.039	1.03	1.005	1.049	1	1.013	1.027	1.015	1.056	1.01	1.039	1.04	1.003	1
May	mean	4.54	4.71	5.7	4.55	4.95	5.3	4.54	5.7	5.26	5.08	5.07	6.19	5.87	4.46	5.51	4.64	5.01	4.82
	SD	1.011	1.025	1.011	1.009	1.075	1.033	1.006	1.042	1.023	1.011	1.053	1	1.043	1.008	1.093	1.034	1.024	1.024
June	mean	4.8	4.75	5.8	5.16	4.94	5.4	4.7	5.78	5.4	5.18	5.14	6.5	6.08	4.52	5.23	4.77	4.91	4.8
	SD	1	1.018	1	1.054	1.06	1.054	1	1.043	1.015	1.02	1.057	1	1.034	1.016	1.114	1.071	1.004	1
July	mean	4.86	4.82	5.94	5.62	4.95	5.47	4.51	5.79	5.45	5.16	5.29	6.45	6.13	4.51	5.42	4.86	4.98	5.13
	SD	1.059	1.024	1	1.198	1.068	1.08	1	1.048	1.026	1.005	1.066	1.044	1.041	1.042	1.076	1.105	1.01	1.039
August	mean	4.66	4.82	6	4.6	5.06	5.71	4.65	5.93	5.53	5.16	5.45	6.43	6.21	4.47	5.48	4.96	5.18	4.86
	SD	1	1.028	1	1.04	1.078	1.053	1	1.034	1.052	1.021	1.078	1	1.037	1.029	1.069	1.094	1.01	1.013
September	mean	4.8	4.79	6.2	5.14	5.11	5.64	4.99	5.86	5.37	5.36	5.3	6.43	6.23	4.6	5.41	5	5.02	5.09
	SD	1.01	1.033	1	1.129	1.099	1.08	1	1.05	1.082	1.04	1.07	1	1.042	1.034	1.086	1.119	1.026	1.012
October	mean	4.35	4.81	6.09	4.26	4.87	5.44	4.43	5.79	5.44	5.39	5.19	6.3	6.08	4.53	5.29	4.87	4.97	4.84
	SD	1	1.022	1	1	1.037	1.067	1	1.066	1.064	1.008	1.077	1	1.037	1.039	1.06	1.054	1.012	1
November	mean	4.34	4.67	5.83	4.36	4.72	5.01	4.36	5.55	5.07	5.26	4.74	6.3	5.79	4.34	5.18	4.66	5.01	4.79
	SD	1	1.019	1	1.009	1.032	1.043	1.009	1.057	1.051	1.053	1.061	1	1.05	1.018	1.062	1.042	1.038	1.048
December	mean	4.38	4.67	5.3	4.42	4.81	5.07	4.36	5.52	5.13	5.15	4.67	6.3	5.68	4.27	5.17	4.59	5.48	4.81
	SD	1	1.019	1	1	1.08	1.04	1.009	1.05	1.029	1.011	1.025	1	1.067	1.004	1.069	1.044	1.149	1

Appendix 2a: Survival probabilities of Atlantic whitefish in different rivers given a completely freshwater habitat choice.

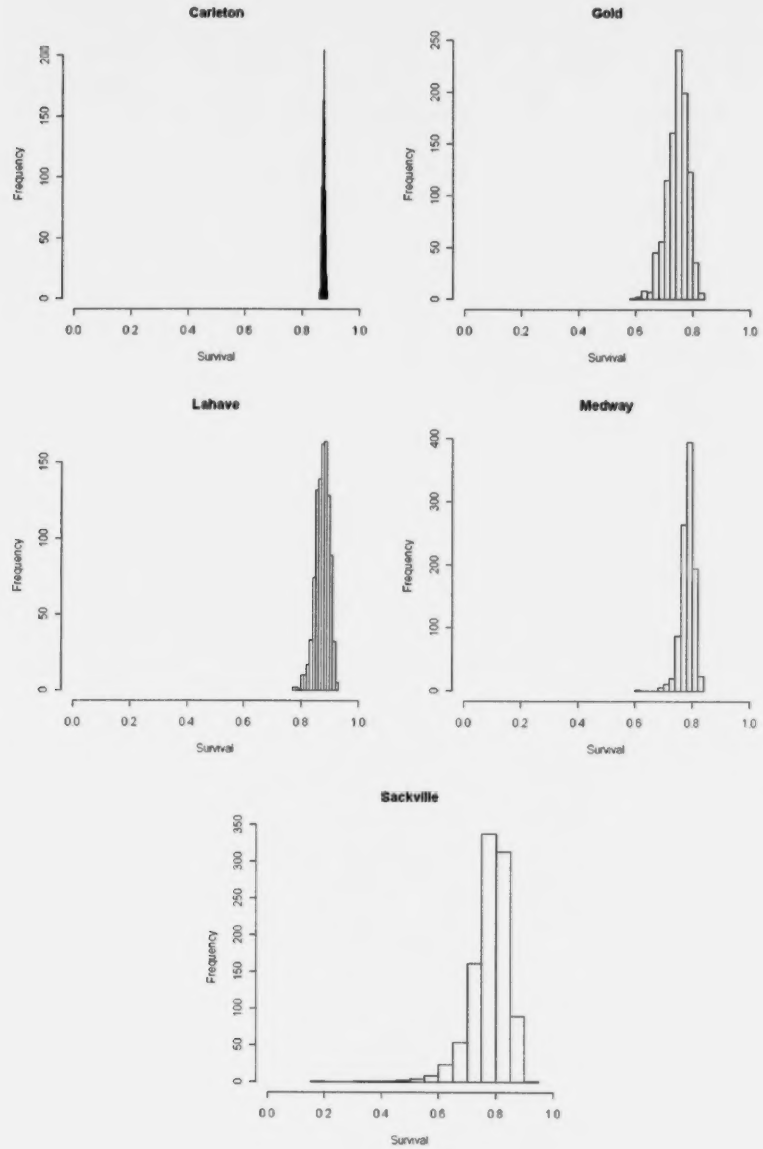
Category 1



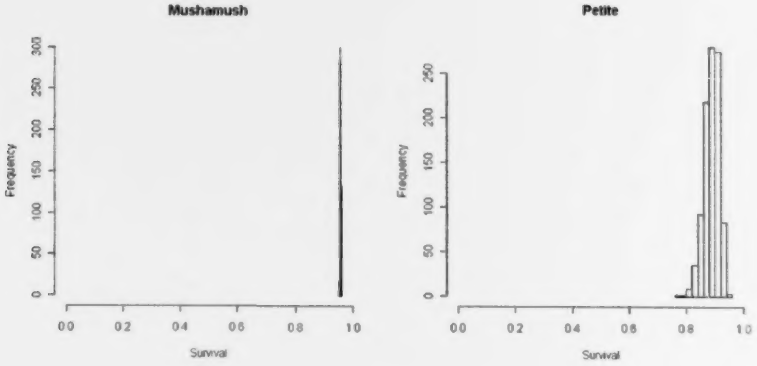
Category 2


Cannan
East
Mersey
Middle
Salmon
Sistiboo
Tusket
Frequency
Survival

Category 3

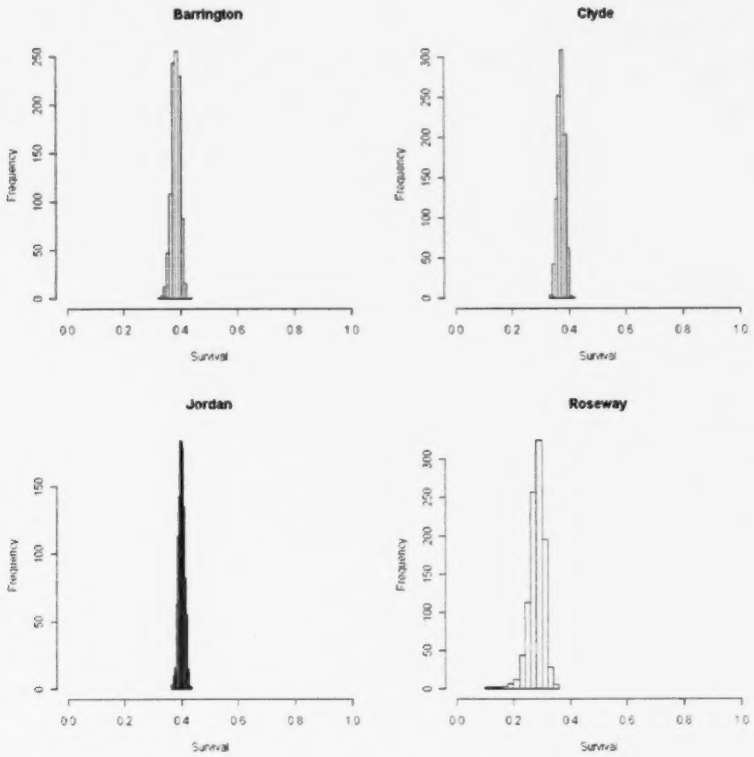

Carleton
Frequency
Survival
Gold
Frequency
Survival
Lahave
Frequency
Survival
Medway
Frequency
Survival
Sackville
Frequency
Survival

Category 4

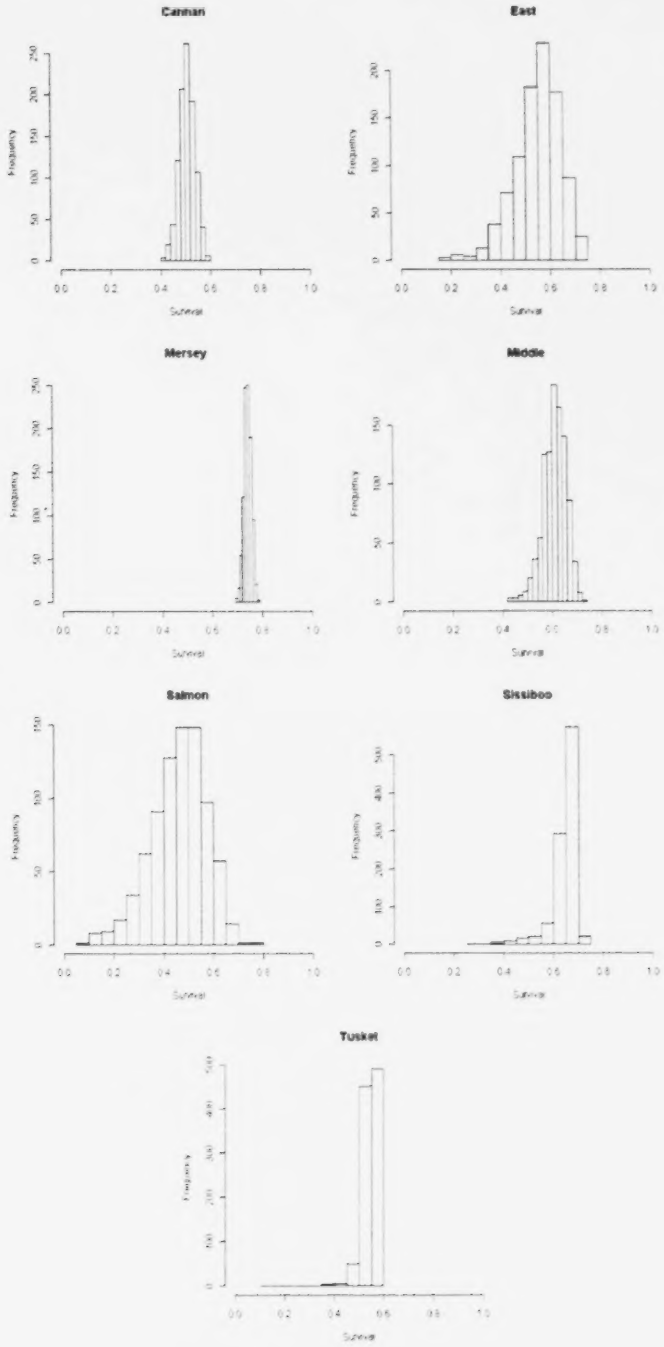

Mushamush
Frequency
0
50
100
150
200
250
300
0.0
0.2
0.4
0.6
0.8
1.0
Survival
Petite
Frequency
0
50
100
150
200
250
0.0
0.2
0.4
0.6
0.8
1.0
Survival

Appendix 2b: Survival probabilities of Atlantic whitefish in different rivers if they made their out-migration as newly hatched larvae.

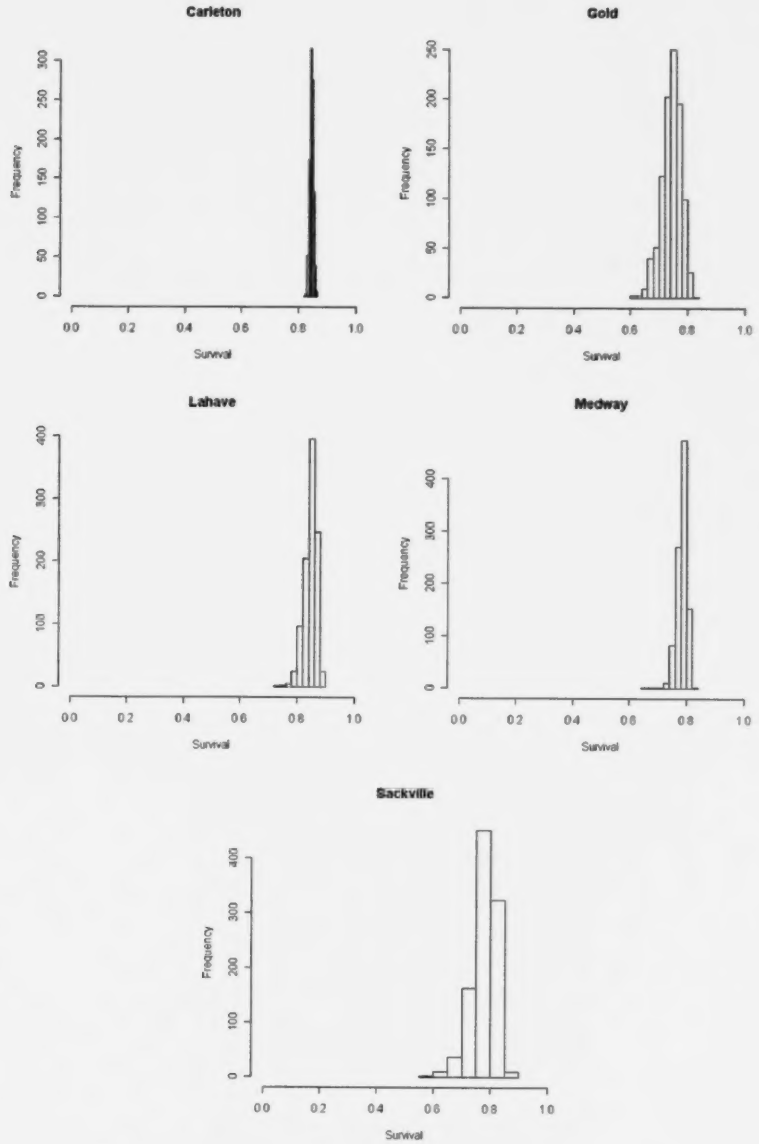
Category 1



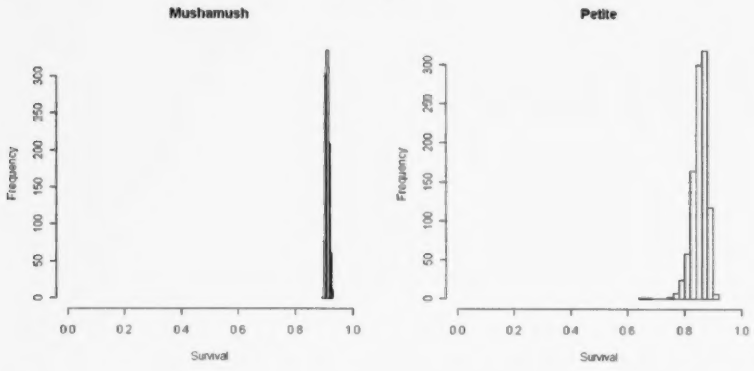
Category 2


Cannan
East
Mersey
Middle
Salmon
Sissiboo
Tusket
Frequency
Survival

Category 3

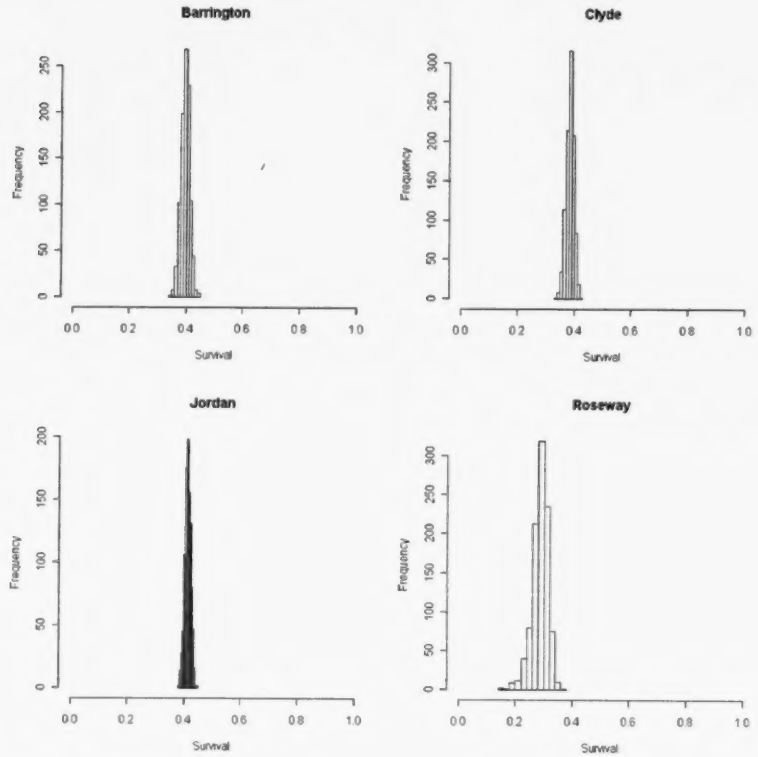

Carleton
Frequency
Survival
Gold
Frequency
Survival
Lahave
Frequency
Survival
Medway
Frequency
Survival
Sackville
Frequency
Survival

Category 4

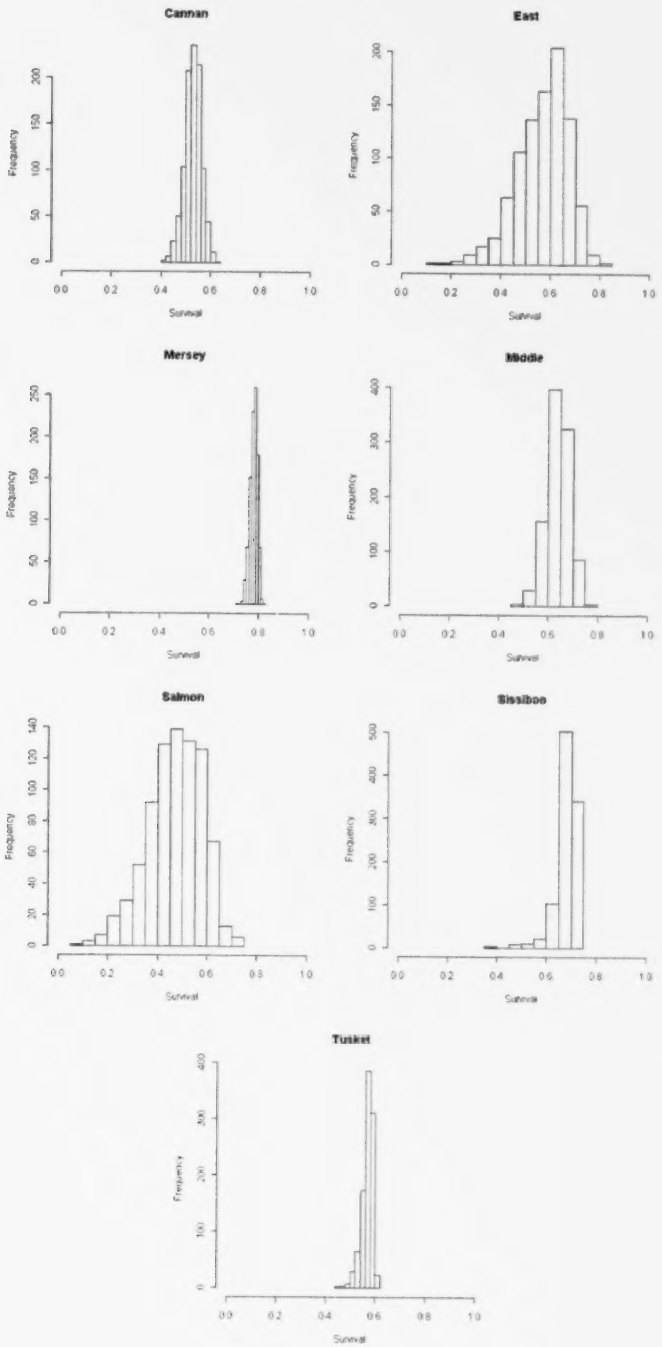

Mushamush
Frequency
0
50
100
150
200
250
300
0.0
0.2
0.4
0.6
0.8
1.0
Survival
Petite
Frequency
0
50
100
150
200
250
300
0.0
0.2
0.4
0.6
0.8
1.0
Survival

Appendix 2c: Survival in pH regimes given an anadromous out-migration after metamorphosing in freshwater.

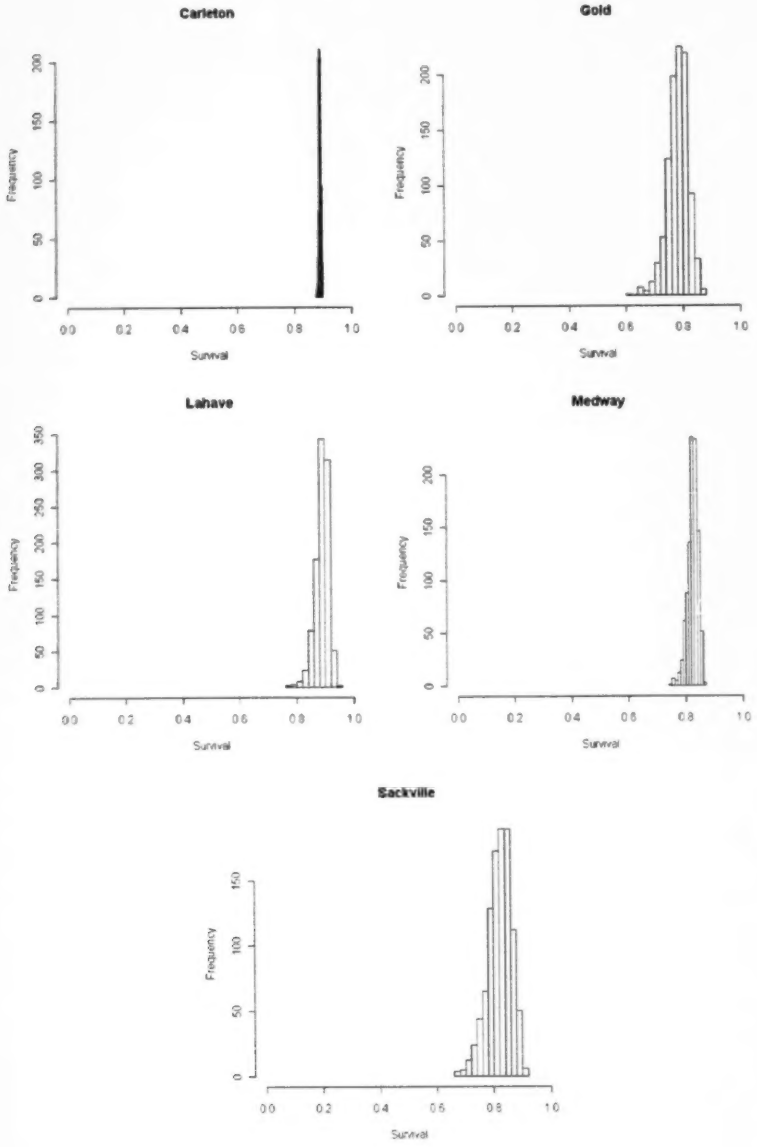
Category 1



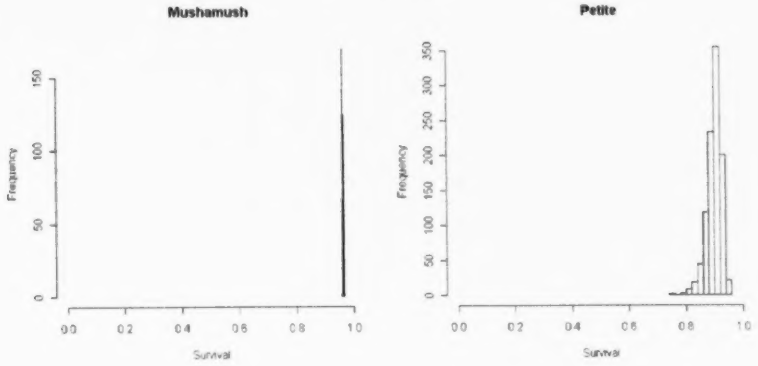
Category 2


Cannan
East
Mersey
Middle
Salmon
Sissiboo
Tusket
Frequency
Survival

Category 3

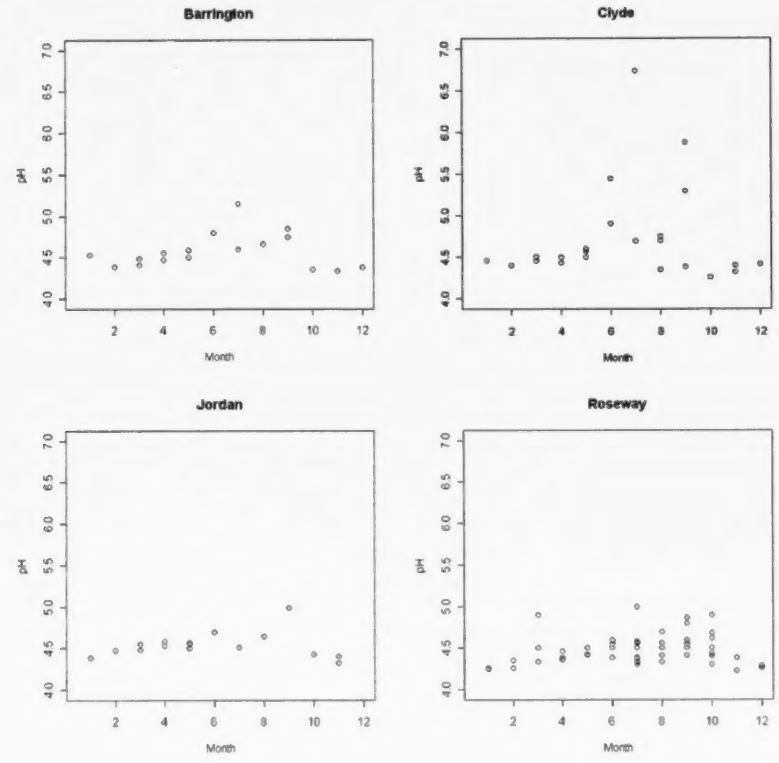

Carleton
Frequency
Survival
Gold
Frequency
Survival
Lahave
Frequency
Survival
Medway
Frequency
Survival
Sackville
Frequency
Survival

Category 4:

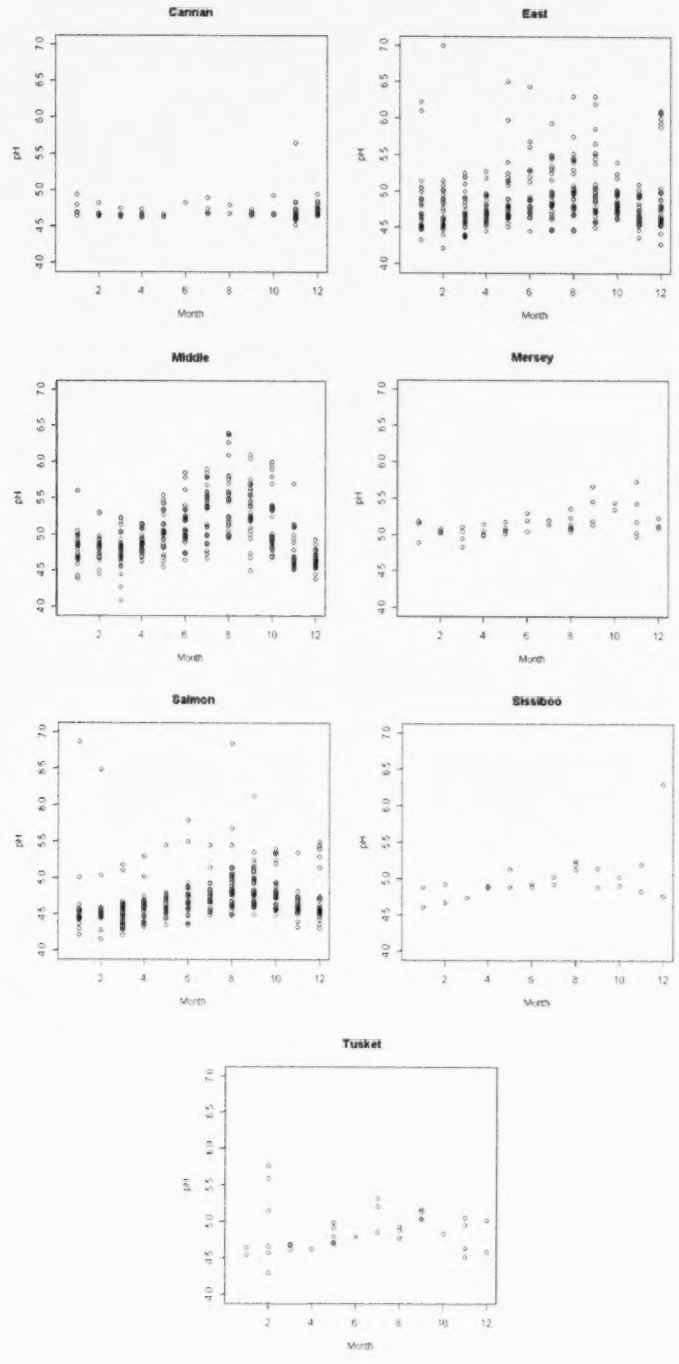

Mushamush
Frequency
0
50
100
150
0.0
0.2
0.4
0.6
0.8
1.0
Survival
Petite
Frequency
0
50
100
150
200
250
300
350
0.0
0.2
0.4
0.6
0.8
1.0
Survival

Appendix 2d: pH profiles of data for each river grouped by pH category.

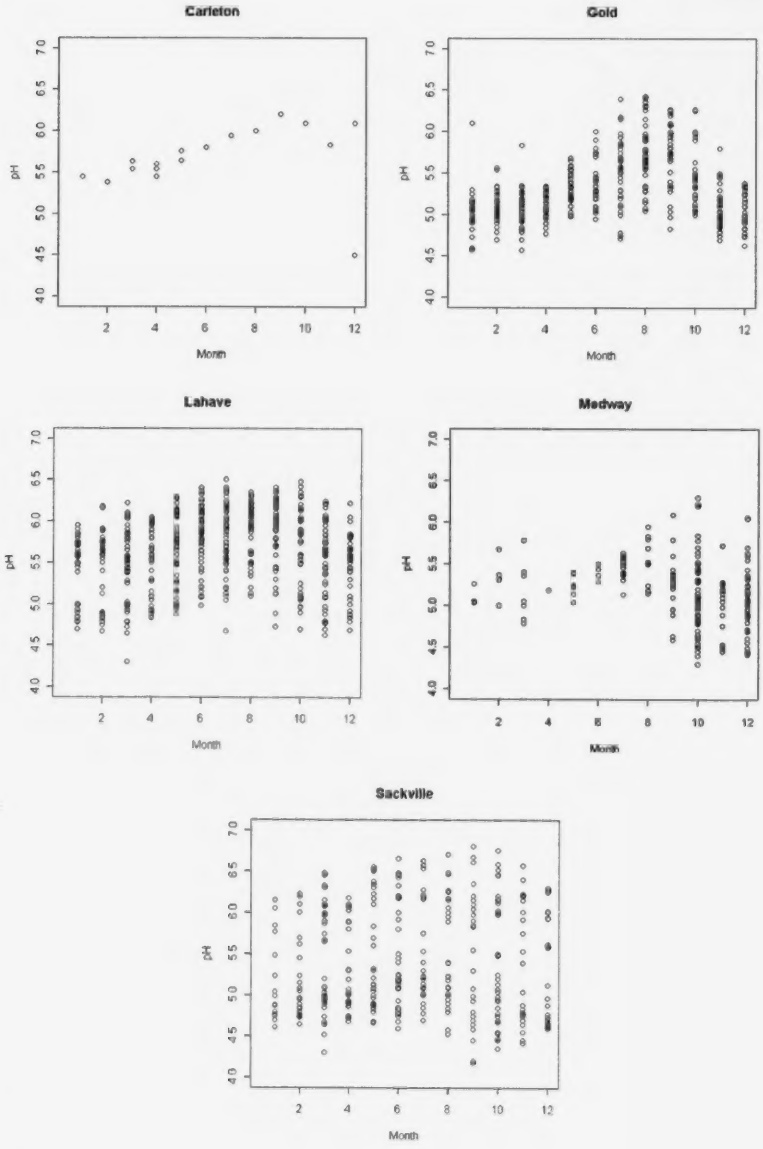
Category 1



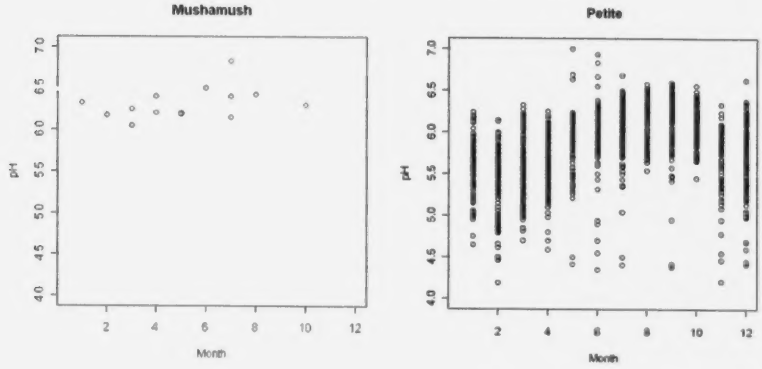
Category 2



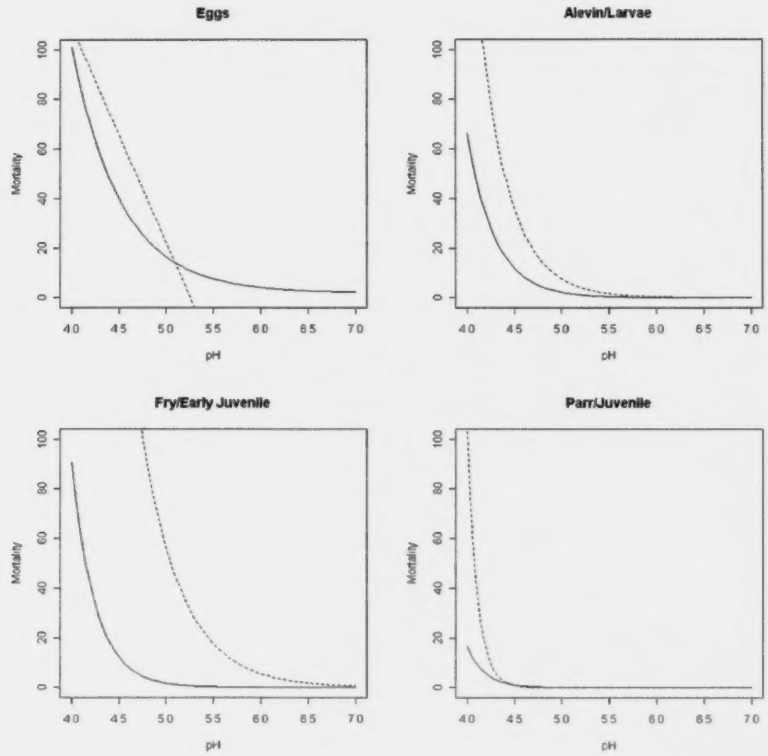
Category 3



Category 4



Appendix 3a: Comparison of regressions from Atlantic whitefish and Atlantic salmon. Atlantic whitefish (solid) and ASRAM models (broken line - Korman et al. 1994) for the effect of pH on different life stages.



Appendix 3b: Comparison of the effect of pH on Atlantic salmon alevins from different experiments. Solid line is mean of Fraser et al. 2008, dashed line is from Buckler et al. 1995 (60d exposure), points are from Peterson and Martin-Robichaud (1986), dotted-dashed line was that used in the ASRAM model (Korman et al. 1994).